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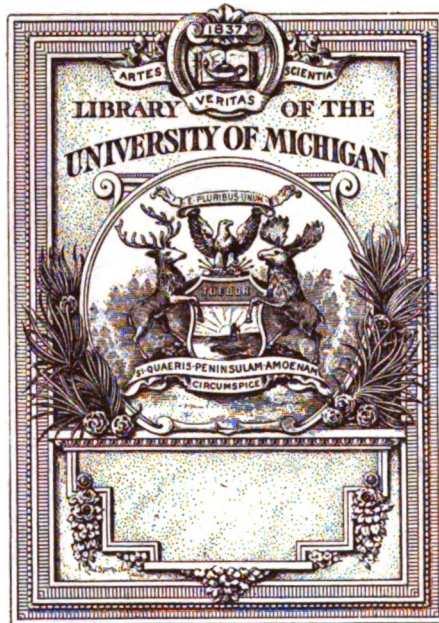
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The Johns Hopkins University

BALTIMORE, MD., U.S.A.

THE
EMBRYOLOGY OF A TERMITE

EUTERMES (RIPPERTII?)

(I)

(Including a contribution to the discussion as to the primitive type
of development, and the origin of embryonic membranes
[amnion] and of the mesoderm in the Insecta)

A DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN THE

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HENRY McELDERRY KNOWER

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THE EMBRYOLOGY OF A TERMITE.¹

Eutermes (Rippertii?).

FIRST PAPER.

(Including a contribution to the discussion as to the primitive type of development, and the origin of embryonic membranes (amnion), and of the mesoderm, in the Insecta.)

HENRY McELDERRY KNOWER.

CONTENTS.

	PAGE		PAGE
Introduction.....	1	Changes in amnion and mesoderm	
Technique.....	2	during this elongation.....	23
General description of egg.....	3	Remaining development up to	
The yolk.....	6	hatching.....	25
Segmentation and formation of the			
first rudiment of the embryo.....	7		
Origin of the mesoderm.....	16	GENERAL.	
Origin of the amnio-serosal fold.....	18	Conclusions as to the primitive na-	
Growth of the amniotic and meso-		ture of this type of development..	29
dermal rudiments.....	19	Origin of the mesoderm of insects..	32
Elongation of embryonic rudiment		Origin of the amnion of insects.....	36
up to segmentation.....	21	Explanatory note.....	53
		Bibliography.....	55

INTRODUCTION.

THOUGH the Termites must be ranked among those insects which have best preserved ancestral morphological traits, their development has not been studied up to the present time. Indeed, it is only within the last few years that we have been enabled to judge of the details of development of any of the primitive forms of insects. The technical difficulties which the investigator of these forms must meet are very great, much time being required to obtain few results, especially in dealing

¹ This paper was accepted as a thesis for the degree of Doctor of Philosophy in the Johns Hopkins University, May, 1896. (See explanatory note at the end.)

with early stages. As Wheeler (26) has suggested, no doubt this has deterred many from working on such material.

Having had the good fortune to secure Termite eggs in ample quantities, I have devoted considerable attention to the development of these interesting insects, in the hope that such a study might throw light on some vexed questions of insect embryology. The present paper must be confined chiefly to the earlier stages of development, the later changes being left to be described at another time in the near future. The eggs were obtained in Jamaica at different times, by myself, and through the kindness of Dr. Hough, Dr. Sigerfoos, and the late Dr. Conant. I am also indebted to Mr. Taylor, of Jamaica, for assistance in obtaining material, and to Dr. William Patten for valuable suggestions as to technique. It is a great pleasure to express my appreciation of the constant kindness and useful criticism and advice of Professor W. K. Brooks, during my work at the Johns Hopkins University.

TECHNIQUE.

My material was fixed with hot water, with cold picro-sulphuric acid (alcoholic), and with hot alcoholic picro-sulphuric, the latter giving the most satisfactory histological results when the acid was promptly washed out after fixation. Specimens fixed in hot water and transferred into 70 per cent alcohol are quite satisfactory.

As Wheeler found in working with Orthoptera, sections through these eggs are very difficult to obtain. Though tedious, Heider's (13) method of painting each section before cutting gives good results. A modification of Dr. William Patten's ingenious method of orienting small objects for cutting is most satisfactory in sectioning these eggs. I feel that my success in handling this material is largely attributable to Dr. Patten's suggestions as to technique and accuracy in methods of study.

In sectioning early stages of the Termite germ-disc, I break off most of the yolk in clove oil, with very sharp needles, under the dissecting microscope, and stick the particle to be sectioned

on a piece of tracing paper smeared with a thin layer of collodion fixative, and ruled with parallel lines by a needle point. The paper is now dipped into xylol and then placed in the paraffine bath. After imbedding, the paper may be stripped off, leaving lines on the surface of the paraffine which give the proper direction for cutting. It is possible in this way to obtain especially thin sections of quite early discs, in any desired plane. Enough yolk adheres to the disc, when dissected off, to make plain its relations to the interior of the egg. In later stages the entire egg is satisfactorily sectioned by the same method, care being taken to prick the side which will lie uppermost in the paraffine bath, to insure penetration. To prevent the yolk from becoming brittle, eggs which are to be sectioned should not be kept in clove oil any longer than is necessary to clear thoroughly.

In studying the egg as an entire transparent object, I have found it best to prick each specimen with a sharp needle under the dissecting microscope; then stain in Grenacher's borax carmine for two days; wash out in cold 70 per cent alcohol, acidified with 20 drops of nitric acid to every 100 cc. alcohol, for three or four days or longer, until the stain remains only in the nuclei; transfer gradually to absolute alcohol, and finally into xylol, which gives whiter and clearer specimens than clove oil.

Eggs for sectioning are usually stained in this way before cutting, though it is best not to wash these out so much, and as I have said, clove oil is then used to clear.

Good surface views of the germ-band at various ages are also obtained by staining the specimen, dissected off from the yolk, in rather strong Delafield's haematoxylin for a very short time.

GENERAL DESCRIPTION OF THE EGG.

There are no special chambers for nurseries, or for the queen, in the nests of this species of *Termes*. (This species has been described from Jamaica as *Eutermes Rippertii*. I shall question this for reasons which will appear at another time. Hence the interrogation mark after *Rippertii* in the title.) On cutting

open a nest, the passages which traverse it in all directions are seen to have no regular arrangement. The walls of the inner passages are thinner than those at the surface. The queen is generally found in the interior of the nest, near its base, surrounded by numerous workers and larvae, and not far from the eggs. The eggs have been collected into heaps, by the workers, as they were laid, and piled up without any apparent system in the passages near the queen. The older larvae also assist in caring for the eggs. It is an easy matter to collect quantities of eggs, since they are quite moist and adhere together in masses, in which all stages of development are to be found, from unsegmented ova to larvae emerging from the egg-membranes. Considerable time must be subsequently devoted to picking out stages from such mixed material.

The unsegmented egg is about 0.5 mm. long by 0.22 mm. in the shorter diameter. It is elongated, larger at one end, and markedly convex on one side.) This shape makes it easy to determine the planes of symmetry from the start, since the enlarged or micropylar end is found to be the posterior pole, while the convex side is the ventral surface. In the course of its development the embryo changes its position by a remarkable process of "revolution," like that described for the Libellulids and certain primitive Orthoptera. This must be kept in mind in speaking of the anterior and posterior poles, and of the dorsal and ventral surfaces. As used above, the micropylar end of the egg is the definitive, as well as the primary, posterior pole, and the convex micropylar side becomes the final ventral surface.

It will appear that the embryonic rudiment when first established, and until shortly after the closure of the amniotic cavity, lies entirely on the convex ventral surface of the yolk. At this period the entire embryo occupies but a small area on this surface, just beneath the micropyles, at its extreme posterior limit, the hind end of the germ-band reaching the posterior pole of the egg.

In the last stages of development the same relative position, lost during intermediate changes, is reestablished by "revolution," so that the posterior end of the embryo comes again to

lie on the convex surface at the micropylar pole of the egg, while the head coincides with the anterior limit of the same surface.

Dr. Hagen (12) described the micropyles of the eggs of this species as follows: "The micropyles of *Termes* eggs have not before been known. Near the upper pole of the egg, on each side, there are four to six flat impressions; viewed in profile they are similar to a soup dish. In the middle of this shallow funnel is a tube of smaller diameter going through the yolk-membrane in the direction of the egg pole."

This description must be modified somewhat; since I have found that the funnels are more numerous, and are grouped on the ventral surface. The chorion is the only membrane penetrated by these funnels. The micropyles are arranged in a semicircle, on the convex face of the egg, near the posterior end. This semicircle is composed of from 12 to 18 funnels, which vary considerably in arrangement. They may be strung out into a single line, curving over the ventral surface, just above the place where the embryonic disc first appears, and extending up on either side toward the dorsal surface. Often the funnels at the two ends of the line are crowded together, while the ventral median ones form a single row (Pl. XXIX, Fig. 1). In some eggs, on the other hand, the funnels of the median ventral row are gathered together, while the lateral openings extend in a line on either side. In surface preparations the rims of the funnels at the mouths may be seen to be slightly corrugated.

Sections of the chorion above the germ-disc, as represented in Pl. XXXI, Fig. 31, cut the micropyles through the wide outer opening, the penetrating canal, or the inner opening.

Dr. Hagen (12) thought he could "see distinctly a bunch of filiform spermatozoa inside the micropyles in some eggs," and that in one case he had succeeded in bringing them out by pressure. I have never found any spermatozoa. Filiform bunches are often seen beneath the chorion. On examination such appearances have proved to be due to various objects, but never to spermatozoa. In surface views of alcoholic specimens, the folds of the vitiline membrane and protoplasmic threads

attached to the chorion, at times might be mistaken for bunches of filiform bodies. When such specimens are crushed, the membrane wrinkles up and looks like bunches of filaments. The eggs from one nest had been attacked by fungus to such an extent that the hyphae, entering the micropyles, had ramified in the yolk and used it up, destroying the embryos. Dr. Hagen could hardly have confused such large objects as these hyphae with spermatozoa. As sperm failed to appear in any of my stained specimens, I am inclined to think that folds in the membranes or strands of protoplasm were mistaken for spermatozoa.

THE YOLK.

My observations on the yolk were made on preserved material. The yolk-mass, thus studied, is composed of a lot of polygonal bodies, which are vesicles containing a homogeneous coagulable fluid. These bodies stain deeply in haematoxylin and carmine, and very considerably in size and shape (Pl. XXXI, Figs. 30-37, *yb.*). The other constituent of the yolk-mass is an oily fluid distributed in small globules throughout the egg, and often found collected in one or two quite large drops. When alcoholic specimens are crushed, the oil globules flow together and run out, leaving the yolk-bodies for the most part intact. In such cases the character of the vesicles can be made out. They are found to vary in structure, part of them being filled entirely with homogeneous coagulable substance quite elastic under pressure.

Many of the bodies, however, differ from this. They contain the homogeneous stainable substance, but also little oily drops, of which there may be only a few or a great number in a vesicle. The greater the collection of these drops, the less is the homogeneous material, so that it is often reduced to a porous substratum for the drops. It is possible to crush such vesicles so that the oil drops escape through the membranous walls. Examined in clove oil or in sections the yolk has quite a different appearance. In these specimens the oily matter is all dissolved out of the yolk, even from the interior of some of the vesicles. Treatment with oils and subsequent heating dur-

ing imbedding often cause numbers of the yolk-bodies to fuse together into large bodies. This may be very marked, large spaces being left in the yolk by the solution of the oily fluids. In sections single yolk-bodies are frequently found riddled with holes, left by the solution of the oil drops formerly imbedded in their substance (Pl. XXXI, Figs. 30, 31, 34, 37, *p. 76*).

I should derive the yolk-bodies in which oil drops have collected from the homogeneous vesicles. The homogeneous (albuminous) coagulable fluid of these bodies apparently becomes transformed into soluble substances which, first forming in isolated drops, finally fill the whole vesicle. In this way the oil globules and free oily fluids of the yolk would appear to arise from the (albuminous?) yolk-bodies, to furnish the growing embryo easily assimilable nutriment. The sections (Pl. XXXI, Figs. 30, 33, and 34) show finely fragmented yolk-bodies lying beneath the embryonic disc. Other sections do not show a similar fragmentation, the solution having been more complete. When yolk-bodies in which drops have collected are prepared for sectioning by the usual methods, chemical changes take place which result in homogeneous fused masses of stainable (albuminous?) substance, and in the solution and removal of the fatty matters.

There is apparently no definite arrangement of the different yolk elements. There is no peripheral layer of protoplasm distinguishable before the formation of a blastoderm. I have found no "segmentation of the yolk" during the early stages of development.

SEGMENTATION AND THE CHANGES IN THE BLASTODERM LEADING TO THE ESTABLISHMENT OF THE FIRST RUDIMENT OF THE EMBRYO.

The first sharply marked rudiment of the embryo is, as in the case of certain of the Orthoptera (*Stenobothrus*, *Stagmomantis*, *Gryllus*, and *Oecanthus*), a relatively small disc of closely crowded cells at one pole of the egg. Since the history of the origin of these embryonic discs has not been worked out, I have studied, with special care, the segmentation

and those changes in the blastoderm cells which result in the formation of the embryonic rudiment on the surface of the yolk.

I have endeavored to answer the following questions: Is the disc formed immediately during the segmentation, by cells wandering directly to the point on the surface where the disc is to appear? Is a blastoderm over the entire yolk surface first formed as a result of segmentation, and then the disc from its cells? Again, if this latter is the method, is the embryo a result of a simple multiplication of the cells of a restricted area of the blastoderm, or is there some other factor present in the formation of the disc?

The answer to these questions requires considerable attention to the earliest stages, but is of much importance to an understanding of the origin of the "under-layer" and of the amnion.

The position of the polar-bodies marks the dorsal pole of the shorter axis of the egg. I have not studied the formation of the polar-bodies from the nucleus, as the few eggs which were at the proper stage did not show the process distinctly enough. To separate from the polar-bodies, the segmentation nucleus moves from the center of the yolk to the center of the dorsal surface. After their formation, the polar-bodies appear as two little rod-like masses of chromatin surrounded by a small quantity of protoplasm, and lie at about the middle point of the flattened dorsal surface of the yolk-mass (Pl. XXIX, Figs. 2 and 3). Later the chromatin breaks into fragments, but the little collection remains visible for a number of divisions.

The segmentation nucleus, on returning from the dorsal surface, lies, just previous to the first division, in the center of the yolk at the intersection of the shorter and longer axes of the egg (Pl. XXIX, Fig. 2).

The first spindle lies at right angles to the shorter axis, so that one of the cells arising from the first division wanders toward the enlarged posterior pole, where the embryo will first appear. The other cell remains near the position that the mother nucleus held (Pl. XXIX, Fig. 3). At the start, then, there is a decided proliferation toward the future embryonic area.

This is brought out better in the following stage, which exhibits two cells in the enlarged end, one on the shorter axis, and one in the small end; that is, there are three nuclei nearer the posterior than the anterior pole (Pl. XXIX, Fig. 4). The cleavage becomes irregular with the eight-cell stage, one or more nuclei dividing before the time for a typical rhythm of divisions. For several divisions there is a slight preponderance of cells in the larger end of the egg. For instance, one egg has four nuclei in this end, one on the shorter axis, and three anteriorly; while another has five in the posterior, and four in the other end (Pl. XXIX, Fig. 5). Generally, during the early stages of cleavage, there are three or four more cells in the larger than in the smaller end of the egg. After five or six divisions, the resulting nuclei have taken positions at about equal distances apart through the yolk. The nuclei are each surrounded by a little mass of protoplasm, and may consequently be spoken of as cells. As far as can be determined, there is no protoplasmic continuity between these cells at this early period. Later, when the embryonic disc begins to appear, continuity is established between its cells; but even then a connection between the blastoderm cells of other regions, or between these and the yolk-cells, is not made out with any degree of certainty. A view of the ventral surface of an egg at this stage shows very well the equal distribution of the nuclei on that side, and the same is found to be true of the nuclei on the remaining surface of this egg (Pl. XXIX, Fig. 6). (*Refer to end of paper, to the explanation of Figs. 4 and 5, in regard to cleavage.*)

Most of the cells have now reached the surface, there being only a few in the yolk which lie at equal distances apart. In properly prepared material, the changes that follow and lead to the appearance of the embryonic disc can be most distinctly traced in entire, transparent eggs studied in clove oil, cedar oil, and balsam. The following description refers chiefly to specimens studied in this way and to sections through certain stages. I have already stated that the various stages are mixed together indiscriminately when collected. The series illustrating the growth of the disc had to be picked out from a great mass of

material. There can be little doubt, however, that a typical series is here figured, for the figures are based on an examination of a great many specimens, and the chief stages are well marked.

Since the first rudiment of the embryo is formed from surface cells alone, the few yolk-cells may be neglected in the description.

Pl. XXIX, Figs. 7-10^a, represent successive changes on the surface of older eggs. The nuclei are found at all points on the surface in the act of dividing, or in pairs just subsequent to division. In the posterior half of the egg this activity becomes especially pronounced, while the nuclei of the anterior half are comparatively inert.

Three surfaces of a somewhat older egg are shown in Pl. XXIX and Pl. XXX, Figs. 11-11^b (ventral, dorsal, and lateral views). As compared with the preceding figures and with the following ones, it is evident that the number of cells in the anterior half of this egg has reached a maximum, which remains constantly about the same in older specimens. The nuclei in this half are few and widely separated. The opposite end, on the other hand, is the seat of active multiplication and change. This is true of the whole posterior end, but it is evident in the three views of the egg before us that the dorsal (Pl. XXX, Fig. 11^a) and lateral (Pl. XXX, Fig. 11^b) surfaces of this half are less crowded with nuclei than is the ventral side. The ventral surface (Pl. XXIX, Fig. 11) exhibits an extensive area of rather closely crowded nuclei, stretching to the extreme limits of the surface posteriorly and laterally. A side view (Pl. XXX, Fig. 11^b) shows a considerable lateral extension of this area, relatively crowded as compared with the rest of the surface.

The posterior half of the surface represented in Pl. XXIX, Fig. 10, exhibits an activity in division and a distribution of nuclei of about the same intensity in its entire extent, forward to the shorter diameter of the egg. A line drawn through the shorter diameter of this figure divides rather sharply an anterior half, with but few widely separated nuclei, from a posterior half, in which the nuclei are more numerous and lie comparatively close together down to the line just drawn. Near the

posterior pole this area is slightly more crowded than near the shorter diameter; but there is very evident activity here, contrasting sharply with the inertia of the cells on the anterior side of the line.

Drawing a similar line across the middle of the older egg (Pl. XXIX, Fig. 11), we find no change anterior to the line. In the region just posterior to this line, extending as far back toward the pole as a second line drawn parallel through the anterior end of the dotted pointer *ca.*, there are fewer nuclei than in a corresponding region of the younger egg (Pl. XXIX, Fig. 10) — by actual count, nearly one-third less than in the earlier stage, or 26 to 36 nuclei. On the other hand, in area *ca.*, Pl. XXIX, Fig. 11, a decided increase in the number of cells is evident, as compared with the preceding stage. The nuclei here are not only one-third more numerous (about 101 to 157), but are much more closely crowded together.

Such a comparison indicates strongly that, in addition to a special activity in cell division within the area *ca.* of Pl. XXIX, Fig. 11, certain cells have actually wandered into this area from more anterior portions of the surface.

If the number of cells in the region anterior to *ca.*, down to the line through the shorter diameter, had remained the same as in the preceding younger stage (Pl. XXIX, Fig. 10), there would have been reason to conclude that this constant number had been maintained, in spite of a multiplication of cells, by a migration back into *ca.* One-half of the product of the divisions of the nuclei might have wandered back into *ca.* from the more anterior region, without disturbing the relations existing in Pl. XXIX, Fig. 10. As it is, the evidence of a migration back into the area *ca.* is much stronger, since an actual decrease in the number of nuclei anterior to *ca.* has been shown; while the increase in the cells of *ca.* is sufficient to allow for this addition from without, as well as for that from a multiplication of the cells already within its limits.

Similar results are obtained from a comparison of dorsal surfaces.

It may be claimed that this method is inconclusive, since the specimen from which Pl. XXIX, Fig. 11, was drawn cannot be

proved to have certainly passed through a stage like that of Pl. XXIX, Fig. 10, having been selected from a lot of eggs in which all stages were mixed indiscriminately. The condition shown in Pl. XXIX, Fig. 11, may have been reached without migration by a more active multiplication of cells in the area *ca.* from the first, the blastoderm anterior to this region remaining comparatively inert. In other words, the center of activity may have been placed more anteriorly in Pl. XXIX, Fig. 10, than in Pl. XXIX, Fig. 11, from the start.

In spite of this possibility of error, I believe the figures do represent successive stages, and that the area *ca.* on the surface of the egg in Pl. XXIX, Fig. 11, etc., has been established, not only by a multiplication in that region, but also by the addition of cells migrating into it from without. This conclusion seems justified by a similar examination and comparison of many eggs in these stages.

Pl. XXX, Fig. 12, is a slightly older ventral surface showing a like extension of the area *ca.*, where more nuclei are now found. Note especially the rather short intervals between the nuclei in the posterior and lateral regions.

Pl. XXX, Figs. 13 and 14, exhibit in ventral and lateral views a further result of the processes just studied.

Comparing Pl. XXX, Fig. 13, with the younger stages in Pl. XXIX, Fig. 11, and Pl. XXX, Fig. 12, the number of nuclei in regions anterior to the area *ca.* is seen to have remained constant, in spite of a multiplication of cells there being demonstrable. Within the former area *ca.* there has been a great increase of nuclei, especially near the center. This is undoubtedly due in part to continued cell division here; but also, as the above observation makes plain, there is evidence of an addition of migrating cells resulting from multiplication in more anterior regions.

Comparing Pl. XXX, Figs. 13 and 14, still closer with Pl. XXX, Fig. 12, additional and striking evidence is found of a further migration of cells from the boundaries toward the center of the former area *ca.*

On rolling the egg figured in Pl. XXX, Fig. 12, the area *ca.* stands out more sharply from the surrounding surface than is

shown in the figure. Near the lateral and posterior boundaries, as well as in the center, the nuclei are about equally distributed and lie rather close together. Turning to the older stage (Pl. XXX, Fig. 13), it is evident that the nuclei in the lateral portions of the same area are fewer than in the younger egg, and nearly twice the distance apart. The egg (Pl. XXX, Fig. 14), being rolled slightly on one side (though not nearly so much so as Pl. XXX, Fig. 11^b, with which it must not be compared), shows this better than Pl. XXX, Fig. 13, in which the convexity of the surface makes it impossible to give an accurate idea of the distribution of the nuclei at the sides. The letters *l.b.d.* indicate a like region in both figures (Pl. XXX, Figs. 13 and 14). It is the portion of the surface lying outside of (lateral to) the position marked by these letters that shows a diminution in the number and a wider separation of the nuclei, as compared with the previous stage.

These changes within the limits of the posterior half of the ventral surface, between the stages of Pl. XXX, Figs. 12 and 13, resulting in a perceptible diminution in the number of nuclei laterally, with an increased crowding toward the center, apparently necessitate an active migration of cells centripetally, coöperating with cell multiplication, to establish the embryonic disc.

Pl. XXX, Fig. 15, is an example of an older egg, showing an extreme concentration of the embryonic disc.

In Pl. XXX, Fig. 18, which represents the ventral surface at a much later stage, the embryonic region, now appearing as a conspicuous and sharply defined circular disc of nucleated protoplasm, hardly occupies one-half of the area formerly marked *ca*. The surrounding cells are few and widely scattered, while the comparatively broad, crowded area in the earlier figures (Pl. XXX, Figs. 12 and 13) has contracted to the smaller, densely crowded, circular embryonic rudiment. There is a marked concentration in the germ-disc visible in passing from the stage shown in Pl. XXX, Fig. 17, to that of Pl. XXX, Fig. 18. Note the concentric crowding of the nuclei along the sides of the disc in Pl. XXX, Fig. 18, as compared with the preceding figure.

A study of sections of eggs passing through these stages apparently confirms what is learned from surface views.

In its early stages the embryonic disc is in cross-section a comparatively broad, flat plate of protoplasm formed by the fusion of its cells, the neighboring cells of the blastoderm being connected rather loosely with the edges of this area (Pl. XXXI, Fig. 30). In reaching its final restricted size in Pl. XXX, Fig. 18, the broad plate of protoplasm, whose boundaries were well defined in an earlier section, has become much reduced in extent. The section of the completed disc (Pl. XXXI, Fig. 31) shows the plate contracted to a decidedly shorter diameter. (The two sections (Pl. XXXI, Figs. 30 and 31) are drawn to the same scale.)

The manner in which the mesoderm arises (described further on), partly by a crowding of cells below from the embryonic area as it becomes defined, is another argument in support of the view here advanced for the formation of the first rudiment of the embryo.

The area of the blastoderm, the origin and gradual concentration of which we have thus traced, will be henceforth spoken of as the embryonic area or germ-disc. Though it might be so called at an earlier stage, it hardly merits the term before reaching the definiteness of outline shown in Pl. XXX, Fig. 18.

The facts here reviewed appear to me to prove that the embryonic disc is not formed directly in the segmentation by cells wandering toward a predetermined point. The evidence indicates also that the disc is not the result of simply active cell multiplication in a restricted area of the blastoderm. The truth seems to be that segmentation results in the establishment of a blastoderm of cells scattered over the entire surface of the yolk, and that then, as these cells increase in numbers, a process of concentration draws many of them together to form an area on the ventral surface, which is the first rudiment of the embryo, the germ-disc. This is shown in the entire series of stages figured, and is brought out vividly by a comparison of Pl. XXX, Figs. 12 and 13. with Pl. XXX, Fig. 18. In Pl. XXX, Fig. 12, the embryonic area spreads over the whole of the posterior half of the ventral surface of the yolk. In Pl. XXX,

Fig. 13, the limits of this diminishing area have drawn well in toward the center and away from the lateral margins of this portion of the ventral surface. In Pl. XXX, Fig. 18, the germ-disc hardly covers one-half of its extent in Pl. XXX, Figs. 12 or 13.

The appearances are not at all what would be expected from a simple cell multiplication in a restricted area. In such a case the growing disc should, it seems, be formed from the coalescence of several areas multiplying around separate centers, or should spread out on all sides as its cells multiply around a single center. As the figures show, the disc is here formed by a steady contraction of a primarily extensive area toward a central point.

The fact that, at even so late a stage as one showing the amnio-serosal fold, the nuclei of the disc are of the same size as those in the surrounding blastoderm, perhaps lends some support to the above contention; since we should expect a rapid multiplication within a restricted area of the blastoderm to produce a mass of cells in that region of smaller size than on the surface elsewhere. In the Termite, during this period, the nuclei of the blastoderm in the whole posterior half of the egg appear to divide with about the same rapidity. The process of concentration, which draws the cells together to form the disc, is accompanied by a steady multiplication of the cells about to be incorporated in it, but the nuclei of the rest of the blastoderm divide also. The position of the embryonic disc is consequently not marked by nuclei smaller than those elsewhere on the blastoderm, in the stages we are considering.

The first rudiment of the embryo is certainly not formed around a number of discrete centers, as is claimed for some decapod crustacea and certain insects. The concentration leading to its first formation is, from the start, most apparent in the posterior portion of the disc. The posterior border becomes sharply defined at an early stage, as the cells draw together in concentric rows from the posterior pole. The lateral edges are next involved; but much later, when the disc is otherwise well outlined and its cells are quite closely crowded, the nuclei of the anterior end have not yet drawn together (Pl. XXX, Figs. 18 and

19). When the amnion is about to close over, the cells of this end have drawn together and become incorporated in the disc.

I cannot determine whether the concentration, in the early stages, is accomplished by the migration of independent amoeboid cells toward the embryonic area, or whether the blastoderm outside this area is from the first a continuous membrane of loosely connected cells which contracts toward the center of the germ-disc. I believe, however, the blastoderm cells beyond its limits to be independent, to a late stage in the formation of the disc.

A less marked concentration of the surface cells has been observed in other insects in similar stages, resulting in a closer approximation of the cells of the embryonic area. Refer to Patten (21), Figs. 1 and 2 of Pl. XXXVI (A), and Fig. 5 of Pl. XXXVI (B), and Wheeler (25), Figs. 63, 64, 66, and 68.

In the Termite's egg, where the embryo is a comparatively small disc when completely established, the concentration to establish this disc is an especially notable process.

McMurrich (18) has discovered a similar method of the formation of the embryonic rudiment in Isopods. His figures, 17-19 and 50-52, show the formation of the germ-band in these crustacea by a concentration of the surface cells toward the ventral side of the egg. He finds an intimate connection between this phenomenon and the formation of an "under-layer," and my observations on the Termite's egg lead me to a similar conclusion for it. Hence the detail in which I have described the early stages.

ORIGIN OF THE MESODERM.

I have studied the origin of the under-layer with especial care, on account of the recent conflicting results of Wheeler (26) and Heymons (14) in regard to its formation in the Orthoptera.

In the Termite there is no gastrula invagination. The under-layer begins to appear at an early stage in the formation of the disc, somewhat earlier than Pl. XXX, Fig. 14, when its cells first begin to be crowded. During this period, at irregular points in the embryonic area, lateral as well as median,

some of the cells are pushed below the surface by the concentration of the blastoderm. Other cells are separated toward the under surface of the ectoderm, by tangential divisions of its nuclei, at various scattered points (Pl. XXXI, Fig. 30).

As these processes continue, the under-layer constantly gains in bulk. Its formation is to be traced back to the concentration of the cells of the disc, and when this has reached the stage represented by Pl. XXX, Fig. 18, the under-layer cells have for the most part collected into a plug projecting into the yolk. From the surface this plug appears as a darkened area of crowded nuclei near the center of the disc.

Preparations of a series of discs, after the under-layer has become thus crowded into a plug, illustrate the growth of this collection of cells. Pl. XXX, Figs. 16-19^a, show, in surface views, the gradual extension of the plug, up to the time when the amnio-serosal fold has grown well forward over the disc.

Sections through these stages and those just preceding and immediately following, taken in connection with what has been learned from surface views, give interesting data as to the formation of the under-layer and the amnion.

Pl. XXXI, Fig. 30, gives a cross-section of the single-layered disc at a stage somewhat older than Pl. XXX, Fig. 13, when it is first definitely outlined from the surrounding blastoderm. There is a crowded appearance of the cells, and some of the nuclei are displaced from the surface and seen wedged below. At various points in the surface layer, at the sides as well as near the middle, nuclei are also found in the act of dividing toward the lower surface, thus adding to the number of cells adhering in the lower layer of the disc.

A cross-section (Pl. XXXI, Fig. 31) of the embryonic area through the region of the plug at the stage (Pl. XXX, Fig. 18), when compared with Pl. XXXI, Fig. 30, cutting the same region of a younger disc, shows that the plug has grown considerably by the gradual addition of cells from the ectoderm and their subsequent multiplication. The mesodermal plug is still in close continuity with the ectoderm.

A sagittal section of a disc of this age (Pl. XXXI, Fig. 32) shows the plug quite distinctly.

Both surface views and sections of these stages agree in exhibiting no gastrular groove. On the contrary, it is as I have stated — the under-layer arises at all points in the germinal disc, as a result of the concentration of this area and of the tangential divisions of its cells. The formation of a mesodermic plug is apparently a further outcome of the concentration. (Consult McMurrich (18) on the formation of the under-layer in Isopods.)

A discussion of the general bearing of these facts on the origin of the mesoderm in insects will be found further on.

ORIGIN OF THE AMNIO-SEROSAL FOLD.

I have devoted much attention to the early history of the embryonic membranes, on account of the general interest their presence excites.

When the amnio-serosal fold is first clearly defined as a fold in sections, it appears from the surface (Pl. XXX, Figs. 19 and 19^a) as a semilunar fold along the posterior border of the embryonic disc, extending forward on either side toward the anterior end. Sagittal sections of this stage make plain that the inner or amniotic layer of the fold is not distinguishable from the ectoderm of the germ-disc, except by its position (Pl. XXXI, Fig. 33). It is of the same thickness as the ectoderm, and its nuclei are arranged in the same layers, inverted. The outer or serosal portion of the fold, on the other hand, is quite different (Pl. XXXI, Fig. 33). This is a thin membrane of much flattened cells with nuclei far apart. This membrane resembles the rest of the extra-embryonic blastoderm of which it is a continuation. (*This evident distinction between amnion and serosa is important, as will appear further on.*)

Figs. 19 and 33 of Pls. XXX and XXXI, though representing the amnion when first appearing as a completed fold, do not exhibit the earliest stage in the formation of the amnio-serosal fold of the Termite.

Several stages before a fold can be made out in sections, its position is outlined on the surface of the disc. When the under-layer plug first appears in surface views, the embryonic disc

is quite sharply marked out, especially on its posterior border (Pl. XXX, Figs. 16 and 17). It is along this border that the amnion is to appear. Pl. XXX, Fig. 18, with the two figures just referred to, shows that, as concentration of the embryonic area proceeds, the nuclei at the posterior end draw together into the disc in concentric rows, which results in a closely crowded semicircle of cells that becomes quite conspicuous in surface views. In Pl. XXX, Fig. 18, this semicircle has become a band of nuclei, much darker than the region of the disc just in front of it, where the nuclei are not so densely crowded.

Sagittal sections of discs in these stages (Pl. XXXI, Fig. 32), younger than that illustrated by Pl. XXXI, Fig. 33, teach that the posterior margin, corresponding to the dark semicircle on the surface, differs from the rest of the disc only in a somewhat greater thickness of the ectoderm. There is as yet no fold in sections.

It is evidently the posterior thickened margin of Figs. 18 and 32, which has folded over in Figs. 19 and 33, to become the amnion.

It will be noted then, in reference to the origin of the amnion, that it is formed with the disc in the same process of concentration, and that it is, at first, evidently merely a specialized portion of the disc before folding forward to become the amnion.

This agrees essentially with the figures which Bruce (6) gave for Mantis (Pl. IV, Figs. 42 and 43); with Patten's (21) description and figures of the Phryganid; with Will's (27) account of the Aphids; and with the results of most observers, though all do not agree in regarding the amnion as a part of the embryonic rudiment.

I have reserved a final section of this paper for a general discussion of the origin of the membranes in insects.

CONTINUED GROWTH OF THE AMNIO-SEROSAL AND MESODERMAL RUDIMENTS TO THE CLOSURE OF THE AMNIOTIC CAVITY.

Preparations of eggs illustrating successive stages in the closure of the amniotic cavity show that this is accomplished by the single semilunar fold growing forward from the posterior

end of the disc. There are no separate lateral folds, nor is there any "head-fold." In a series of specimens represented in Figs. 19-24, Pls. XXX and XXXI, the membranes are found extending further and further anteriorly over the disc. In Pl. XXX, Fig. 23, the amniotic cavity remains open in only a single spot at the anterior extremity of the disc, the closure of which opening, in Pl. XXXI, Fig. 24, completes the process.

A series of sagittal sections, like that shown in Pl. XXXI, Figs. 32-35, gives a better idea of what has just been pointed out in the surface figures. (The nuclei in the resting stage in this series of figures are represented in solid black for the sake of clearness. They resemble those in Pl. XXXI, Figs. 30 and 31, being large, vesicular, and containing fragmented masses of chromatin.)

Pl. XXXI, Fig. 32, already referred to in a previous section, exhibits the appearance and relations of the amnio-serosal and mesodermal rudiments when first well established. The mesodermal collection of cells lies under the anterior half of the embryonic disc. It does not extend beneath the extreme anterior end, and is still rather intimately associated with the ectoderm from which it arose. Behind this mesodermal plug, and between it and a posterior thickening of the ectoderm (already indicated as the first rudiment of the amnion), is a thinned region of the disc with only one layer of nuclei, corresponding to the lighter portion of the surface view in a like position. Note the immensely enlarged yolk-cell nucleus as compared with one of the mesoderm.

In Pl. XXXI, Fig. 33, a section of the stage (Pl. XXX, Fig. 19), except for an increase in the size of the rather loose mesodermal plug (due partly to a continued migration from the ectoderm, as indicated by the direction of the spindle of the dividing ectoderm nucleus anteriorly, and by the crowding of the cells in the lower layers of the ectoderm), the most striking change is a bending forward of the thickening, marked amnion in the preceding stage, to form a fold. The bend takes place in the thin, single-layered portion of the disc. The serosal cell posteriorly is much flattened, and is drawn forward by a very slender thread of protoplasm. It is interesting to observe, in

this section and the following ones, fine protoplasmic processes running out from the ectoderm. In some instances I have traced such threads out to the chorion and into the micropylar funnels.

As the cells of the amniotic fold have multiplied, it has bent well forward in the next figure (Pl. XXXI, Fig. 34). Its cells form a thick mass and are arranged in two layers. Posteriorly it passes into the ectoderm through the thinned region pointed out in the former stage. The flat serosal cells lie superficially drawn forward with the amnion. The mesodermal plug is more sharply defined from the ectoderm, its cells lying loosely together in the former position and dividing in places.

When the amniotic cavity is finally closed completely (Pl. XXXI, Fig. 35), as in Pl. XXXI, Fig. 24, from the surface, the resemblance between the amnion and the ectoderm is most striking. The cells of both are arranged in two layers and divide in a similar manner. The serosa is now a very thin membrane of large, flat cells, stretching over the embryo and enclosing the yolk. Its nuclei are found, from now on, in resting condition, with one or more nucleoli and granular looking chromatin. They divide seldom. The mesoderm is now sharply separated from the ectoderm, and from this time the separation appears to be maintained. A few mesoderm cells have pushed back to the extreme posterior end of the embryo. At the anterior end the former relations remain unchanged. The yolk-cell nuclei are of remarkable size and have apparently remained undivided from an early stage.

GROWTH OF THE DISC-SHAPED EMBRYONIC RUDIMENT INTO
AN ELONGATED GERM-BAND UP TO THE TIME
OF ITS SEGMENTATION.

Comparing Pl. XXXI, Fig. 24, with figures of earlier stages, it is evident that considerable change has taken place in the shape of the embryo. The disc has now grown larger. It is about twice as long as broad, and while the posterior end is enlarged and rounded, the anterior extremity is rather pointed. The cells of this disc and of the amnion have become much

smaller by repeated divisions, while those of the serosa are now comparatively very large, having before this practically ceased to divide. This transparent egg also shows the few large yolk-cells, seen better in sections.

The growth of the embryo, from the time when the amniotic cavity is completely closed, is chiefly at its posterior end. The hind end of the embryonic band pushes back over the posterior end of the yolk-mass, just beneath the serosa (Pl. XXXI, Figs. 25, 27, and 29), while the head end remains fixed. (In some exceptional eggs the embryo is found out of its usual position, slipped forward or backward.) This growth continues for some time over the posterior pole, no marked change being apparent superficially, except an increase in length and breadth. The anterior end, however, becomes gradually less pointed.

A germ-band slightly older than that shown in Pl. XXXI, Fig. 24, while not yet one-half the length of that in Pl. XXXI, Fig. 26, would have already acquired a square, broad anterior end, as in the later stage.

The embryo in Pl. XXXI, Fig. 26, is not in the usual position at this period, some few eggs thus exhibiting the germ-band entirely on the ventral surface, and giving its shape and relations better than can be shown by drawing an embryo dissected-off from the yolk. Pl. XXXI, Fig. 27, represents in side view this same stage, as it is found usually, with the few exceptions just noted.

The germ-band now continues to push back around the yolk-mass, until about one-third up on the flattened dorsal side of the egg, when the embryo forms a *U*-shaped figure, lying over the enlarged end of the yolk (Pl. XXXI, Fig. 29). At this time the band is still unsegmented. Posteriorly it terminates in a rounded extremity. The anterior end has in the mean while undergone considerable change. From being a narrow-pointed tip to the band (Pl. XXXI, Fig. 24), it first gradually widened into a square end (Pl. XXXI, Fig. 26, and stages between this and Pl. XXXI, Fig. 24), and finally spread out over the yolk anteriorly and laterally, until now (Pl. XXXI, Figs. 28 and 29) this region has become the most prominent part of the embryo. Anteriorly, just in front of the point where the mouth is to appear,

the cephalic region is slightly emarginated. On either side it extends up on the yolk as a broad lobe with rounded borders. Such is the appearance of the embryo just before segmentation. (*See also next section for a description of Pl. XXXI, Fig. 28, of this stage.*)

CHANGES IN THE MESODERM AND AMNION DURING THE
ELONGATION OF THE GERM-BAND BEFORE
ITS SEGMENTATION.

Pl. XXXI, Fig. 36, is a sagittal section through a stage in the elongation of the embryo, slightly older than that of Pl. XXXI, Fig. 24, when the anterior end has broadened and become square, as in Pl. XXXI, Fig. 26. Compared with Pl. XXXI, Fig. 35, this whole embryo is decidedly longer. The amnion appears thinner, its cells are becoming arranged in a single layer, especially at the anterior end. As the germ-band has grown posteriorly, the mesoderm has multiplied by a division of its own cells and followed back, not quite so rapidly as the ectoderm, becoming a flattened pad of cells beneath this layer. (The mesoderm cells are well seen as a flat layer beneath the entire width of a germ-band of this age dissected-off and stained for a surface view.) The mesoderm extends no further forward than in section, Pl. XXXI, Fig. 35, but the ectoderm of the anterior end of the embryo has pushed out in front to a slight degree.

Pl. XXXI, Fig. 25, is a side view of an egg of about the same age as that sectioned in Pl. XXXI, Fig. 36. The embryo occupies a peculiar position for one of this stage, ordinarily being found on the ventral surface as shown in the younger egg (Pl. XXXI, Fig. 24). It appears to have slipped back into the exceptionally large space between the chorion and yolk. It gives a good idea of what is shown in the section, Pl. XXXI, Fig. 36, just described. Note the inflated amniotic cavity. The amnion is seen partly in optical section where it passes into the ectoderm posteriorly, and anteriorly where it is drawn out into a thin membrane. On its surface the cells form a mosaic. The mesoderm cells lie loosely beneath the

thick ectoderm and, in this case, form an especially large mass under the posterior end of the band.

Turning to Pl. XXXI, Fig. 37, we find several important changes. It is a section of the stage in Pl. XXXI, Fig. 27, before the appearance of cephalic lobes. The embryo now forms an elongated band bent over the posterior pole of the egg. The mesoderm has followed the growing posterior end and has become arranged in a thinner layer. Its anterior cells appear to have retained their primary position, as in the preceding stage, but the greater mass of mesoderm has been carried back with the elongating ectoderm, leaving only a single layer beneath the middle of the embryo. This growth of the mesoderm is, I believe, accomplished independently of the ectoderm, by a multiplication and rearrangement of its own cells. There is still a sharp division between the two layers. The growth seems to be more active at the posterior end, while the middle region appears to be pulled out, as it were, the anterior end remaining stationary. The size of the yolk-cells still precludes a later origin of entoderm from these. There is no trace of entoderm up to the time of the segmentation of the germ-band. The ectoderm just in front of the anterior limit of the mesoderm has grown further forward than in the preceding section (Pl. XXXI, Fig. 36). This anterior extension of the ectoderm will continue in later stages, and give rise to the cephalic lobes.

The effect of the backward elongation of the germ-band on the amnion, whose cells are now apparently multiplying but seldom, is well shown in the section before us. Posteriorly it still retains to a slight degree the character of the ectoderm, though much thinned out. Anteriorly the amnion has been stretched out by the pull from behind into a very thin membrane of flattened cells. I have found but few dividing nuclei in the later stages of the amnion, the membrane appearing to be stretched rather than to actively grow. This is beautifully seen in surface preparations, where the amniotic cells, now much larger than those of the more rapidly multiplying ectoderm, stand out in bold relief, lying closer together posteriorly.

The oldest stage of the germ-band just before segmentation is dissected-off from an egg like that in Pl. XXXI, Fig. 29,

and drawn in Pl. XXXI, Fig. 28. It is flattened out with the under (or yolk) surface uppermost.

This embryo exhibits a uniform ectoderm, with cells somewhat more closely crowded in the cephalic lobes. Along the borders of these expansions this crowding is greatest. At the extreme front end of the band, in the median line, wedged in between the lateral lobes, there is a small triangular area of ectoderm, in some preparations much more distinctly shown. Cells of the amnion are seen at the edges of the germ-band. The under, mesodermal layer is shown in such preparations very beautifully. Its cells being differently shaped from those of the ectoderm, lying more loosely, and at the same time staining rather more intensely, the entire layer stands out with remarkable distinctness. A larger collection under the posterior end of the band is apparent, as was shown in sections of the younger embryo (Pl. XXXI, Fig. 37). Passing anteriorly the cells become more scattered. Only two or three cells have wandered forward into the cephalic lobes — the anterior end of the mesoderm being fixed at the base of this region. Here there is a little collection, on either side, under the posterior ends of the cephalic lobes.

Graber's (9) preparations of the germ-bands of *Stenobothrus variabilis*, removed from the yolk in like manner, make a similar picture. His Fig. 76 of Taf. VI represents a stage which may be compared with my Pl. XXXI, Fig. 28, for the Termite, though the cephalic lobes are not so broad in *Stenobothrus*. In the Termite the mesoderm does not lie so evidently along the middle line, but forms a flat layer extending nearly to the edges of the band. The earlier germ-bands of the Termite have a shape somewhat different from those of *Stenobothrus* (Graber (9), Figs. 74 and 75), and here again the mesoderm is not so markedly on the middle line.

GENERAL SKETCH OF THE DEVELOPMENT FROM THE FIRST APPEARANCE OF SEGMENTS UP TO HATCHING.

Before proceeding to a discussion of the phenomena which have been described, I shall trace the remaining course of

development briefly, referring to the series of diagrams on Pl. XXXII for the general characteristics necessary to an understanding of this special study. A complete series of figures of the later stages will be published in the near future.

The first traces of segmentation and appendages appear, suddenly, a little later than the last stage described, where the germ-band had become a *U*-shaped cap over the posterior end of the yolk-mass (Pl. XXXI, Fig. 29). At this stage the antennae have just become evident as backward processes of the cephalic lobes, post-oral in position. The first maxillary and first thoracic are more distinct than the other anterior segments, which are however outlined. The "tail-piece" is long and unsegmented. The anterior segments through the first thoracic have therefore arisen almost simultaneously. There are no macro-somites described by Graber (8) and (9) for *Stenobothrus* and other forms.

Later embryos exhibit a progressive increase in the length and complexity of the germ-band.

When the hind end of the band has pushed forward along the dorsal surface of the yolk almost to the anterior end of the egg, three additional segments have been added. These are the two posterior thoracic segments and the first abdominal, and they are added successively from before back; since I have embryos in which the first thoracic is the last segment distinguishable, others with an indistinct second thoracic behind this, and yet a third lot with three distinct thoracic and an indistinct abdominal segment. In older embryos more abdominal segments are added behind. A "tail-piece" of unspecialized (ecto- and mesoderm) material is found at the end of the band during this process, the abdominal segments being successively differentiated from its anterior edge. (*See final section of this paper and final plate.*)

Graber's (9) beautiful figures of the development of the Orthopteran, *Stenobothrus*, would serve fairly well, in most respects, to illustrate the general features of the growth of the germ-band of the Termite from a disc-like rudiment to an elongated, segmented embryo at the period of "revolution." This process was not observed by Graber in *Stenobothrus*. It

should be remembered, though, that the Termite's germ-band exhibits no "macro-somites" of Graber, and that the disc lacks the prominent gastrula groove of *Stenobothrus*.

As a whole, the resemblance between the Orthopteran and the Termite during the embryonic stages is striking.

A stage corresponding to that figured by Brandt (3), Fig. 11, for *Calopteryx* is reached, with the appearance of the mouth and the labrum, and the subsequent folding of the head up from the surface of the yolk. At the same time the segments and appendages have become more prominent.

The embryo, unlike the *Libellulid*, is not immersed in the yolk. (See Pl. XXXII, this paper, also Korschelt and Heider (17), figures on pp. 774, 776, and 777.)

In the Termite, when the germ-band has grown along the dorsal surface of the yolk to the anterior end of the egg, the posterior portion of the abdominal region sinks slightly into the yolk. As the embryo continues to elongate, this bend in the abdominal region becomes more marked, the tail-end of the band coiling ventrally into nearly a complete circle. (See diagrams, Pl. XXXII.)

This caudal flexure is a very characteristic phenomenon. It occurs in many insects and is much like that of the *Libellulid*. (See Korschelt and Heider (17), figures on pp. 774, 776, and 777.) I cannot explain it. It certainly appears to take place here (as in the *Libellulid*), without being necessitated by any combination of mechanical forces that can be stated.

The formation of this flexure has furnished me with a warning, and a good example of what at first sight appears to be a simple mechanical process, but proves to be a phenomenon not so readily dismissed. In many specimens, a very plausible explanation of it seems to be the resistance offered to the posterior end of the elongating germ-band by the chorion, lying at right angles to its course at the anterior end of the egg. This will not serve as an explanation, however, since in many preparations the flexure occurs before the anterior end of the egg is reached (as in the *Libellulids*). It is clear in one instance, at least, that the tail end of the embryo might grow back on the surface of the yolk around the anterior pole, as in

some insects. There was no caudal flexure in this specimen, the hind end of the band turning part way over the pole.

At the completion of the elongation of the embryo the appendages have become quite long. The head is enlarged and globular. The first maxillae are tri-lobed, and the second pair less markedly so. In the abdominal region ten well-marked segments have become established, each with a distinct pair of appendages. No appendages are figured by Brandt on the abdomen at the corresponding stage of *Calopteryx*, and none of the figures of Graber to which I have referred exhibit such well-marked rudiments in that region.

From this stage until "revolution" the embryo undergoes but little change externally, though the sides of the band grow dorsalward, and the appendages elongate considerably.

"Revolution" is accomplished as described and figured by Brandt (3) (also see Korschelt and Heider (17), figures on p. 777) for the *Libellulid*. The amnion and serosa fuse into a single membrane at one point, only to tear open over the ventral side of the embryo and retract dorsally, to finally form the "dorsal-organ" at the back of the head (stages *O* and *P*, Pl. XXXII). The head of the embryo now slips up along the ventral surface of the yolk to the anterior end of the egg, while the tail end comes to lie beneath the micropyles at the opposite end (see diagrams, Pl. XXXII). The ventral surface of the embryo is now entirely on the micropylar, ventral side of the egg, as was the case until after the closure of the amniotic cavity. The embryo has, therefore, returned to the orientation of its first rudiment, the germ-disc.

The remaining processes, up to hatching, consist in the closure of the body along the dorsal mid-line, the completion of the appendages, and the continued development of the internal organs. In the stages following "revolution," the embryo increases so greatly in bulk that, just before it leaves the egg, this has become distended to a remarkable size as compared with the unsegmented egg.

GENERAL CONCLUSIONS AS TO THIS TYPE OF INSECT
DEVELOPMENT.

Korschelt and Heider's recent text-book (17) contains an argument for a modification of Will's and Wheeler's well-known theory of a connection between the "invaginate," "immersed" type of development exhibited by the Libellulids and some Hemiptera, and the type followed by myriopods.

On p. 775 (17) we find: "The invaginate type is best seen in the Libellulids, which represent the direct connecting link (Anschluss) with the phenomena exhibited by the myriopods, and hence must be regarded as the more primitive type." Again on p. 787 (17): "Wir haben oben gesehen dass bei den Myriopoden bei fortschreitenden Längenwachsthum des Keimstreifs derselbe in seiner Mitte eingeknickt und in das Innere des Eies versenkt wird. In dieser Einsenkung, welche wir uns zunächst durch das räumliche Missverhältniss zwischen dem langgestreckten Keimstreif und der rundlichen Eiform entstanden zu denken haben, werden wir (wie dies schon Graber andeutete und Will neuerdings ausführlicher begründet hat) den Ausgangspunkt für die Entwicklung des invaginirten Keimstreifs der Libelluliden zu suchen haben. Wir werden demnach für die Entwicklung des Insecten Keimstreifs die Form der Invagination als die ursprüngliche betrachten." This account is apparently based on Heider's (13) discussion of the subject in his monograph on *Hydrophilus*. It is a modification of Will's (27), also Wheeler's (25) theory, against which in its original form Graber (9), in a more recent paper than the one referred to before, brought forward strong objections.

Since the publication of the text-book of these two authorities on insect embryology, further investigation has shown, that besides *Oecanthus* which is mentioned in it, a number of Orthoptera, as well as the Termite (which is strikingly orthopteran), exhibit developmental phenomena similar to those of the Libellulids. It now seems evident that there are no grounds whatever for regarding the method of development followed by this latter group as at all more primitive than that observed for

Oecanthus, Gryllus, or the Termite. These forms should be looked to for a connecting link, if one exists (on this question refer to the discussion of the origin of the amnion in insects in the last division of this paper), between the phenomenon of "doubling-up," exhibited by the myriopod embryo, and the formation of an amnion in the Pterygota.

There is much reason for believing the development of the Libellulid to be secondary, since the embryo is of the "immersed" type.

A. A superficial germ-band is generally characteristic of Arthropods, and when we find one sunken into the yolk, there is cause to believe this position has been assumed secondarily. Among the insects, most forms (and especially the Orthoptera and Termites) agree in having superficial embryos. The exceptions are rather marked, and are found among the Lepidoptera, Hemiptera, and Libellulids. In the Lepidoptera, as in the Myriopoda, the "immersed" position is admitted to have been secondarily derived from the superficial for protection, nutrition, or some other unknown cause. It appears to me most probable that the same is true for the Libellulids and the Hemiptera, with inner germ-bands.

Hence I should regard the superficial embryos of the Orthoptera and the Termite as more typically primitive for insects.

B. A striking character of the development of the Termite is the small size of the first rudiment of the embryo, the germ-disc, when compared with the definitive length of the embryonic band. The primary rudiment must elongate through the whole length of the egg, and add successively all the segments of the body before the embryo is fully formed. This is equally noticeable in the case of some of the Orthoptera, but is less pronounced in most insects, particularly among the more specialized forms of the group. In these there is a tendency toward a formation of the embryonic band in its full extent from the start.

Now it seems to me that the Termite and the Orthoptera, with a superficial embryo beginning in a disc which must elongate considerably to attain the definitive number of segments, have adhered most nearly to the typical method of development for Arthropods, and probably best represent the embryonic develop-

ment of the ancestral insects. The facts of the development of the Crustacea, Palaeostraca, the Arachnids, and the Myriopods (see Korschelt and Heider (17), p. 741) show a similar disproportion in size between the primary rudiment and the definitive segmented adult. This may be illustrated from the Arthropods by referring to the growth of a Nauplius into its adult form. A similar method of growth is found in the development of the Annelid from the Trochophore, where also growth is uncomplicated by the presence of yolk. I do not mean to raise any question of homology between the primary disc-shaped rudiment of the insect embryo, and either the Nauplius or the Trochophore, but to point out that a certain few insects (Termites, etc.), otherwise primitive, have retained a method of growth (see closing paragraphs of this paper) fundamentally similar to that followed by other segmented forms. In most insects, and particularly in the more specialized forms, the formation of a segmented embryo is more direct, a rather long germ-band being established from the first (and, as I take it, precociously), of more nearly the definitive length of the embryo. (*Note that Graber's (9) classification of germ-bands is not here accepted.*)

C. *These primitive forms (Orthoptera and Termite) are also characterized by another peculiarity of interest in the present discussion. The amnion arises very early and completely covers the embryo soon after its appearance as a small disc.* We do not know with certainty to what need of the embryo the amnion responds, but we are not surprised to find it in its most primitive condition in the very forms under consideration, which are primitive in so many other morphological characters. I believe this is the case, and that *insects, in which the membranes become prominent and cover over the embryo comparatively late in its growth, represent a secondary condition.* If, as is generally supposed, the amnion arose as a protection for the germ-band against mechanical injury or too rapid evaporation, or as a sac, to receive accumulated waste products, as Wheeler (25) suggested, it would have been a great advantage for it to appear in the ancestral Pterygota at the earliest possible moment in the growth of the embryo. This moment occurs when the first rudiment of the embryo, the germ-disc, is established and about

to grow into the elongated segmented embryo. From this time a superficial germ-band would be constantly exposed to the dangers mentioned. Hence the invagination, at this period, of a part of the disc, resulting in the formation of the amnioserosal fold.

The Termite and some of the Orthoptera (*Stenobothrus*, *Gryllus*, etc.) have best retained this method of the formation of the amnion.

In other Orthoptera, the Libellulids, some of the Hemiptera, and many other insects, the ancestral history is not so well preserved. In these the amnion no longer closes over at the earliest possible stage. Wheeler's figures of the germ-bands of *Blatta* and *Doryphora* (25), Graber's of *Lina* (9), Heider's of *Hydrophilus* (13), and Weismann's of *Chironimus* (24) illustrate its usual late closure.

The Libellulids and some of the Hemiptera retain to a decided degree ancestral characters, but the much-retarded closure of the amniotic cavity, and the presence of the so-called secondary "head-fold," together with the marked secondary "immersed" position of the germ-band, render these forms less typical examples of the probable primitive method of development. (*Refer to the discussion of the origin of the amnion in insects, in the last division of this paper, for further consideration of these questions.*)

THE ORIGIN OF THE MESODERM IN INSECTS.

Recently the origin of the under-layer in what are regarded as the most primitive insects, the Orthoptera, has been carefully studied by two well-known investigators who have reached quite contradictory results.

Wheeler (26), in his "Contribution to Insect Embryology," has devoted considerable space to a review of the question. His conclusion is expressed in these words: "It follows from the observations here recorded, fragmentary as they are in many respects, together with Graber's observations on *Stenobothrus*, that the Orthoptera can no longer be regarded as *hors de ligne*, so far as the formation of their germ-layers is concerned. In

all the families of the order, save the Phasmidae, an invaginate gastrula has been found, and there can be little doubt that the investigator who is so fortunate as to study embryos of this family will find in them essentially the same process of germ-layer formation. The view is now pretty generally held that in the Insecta both mesoderm and endoderm arise from a median longitudinal furrow (the former layer throughout nearly the entire length, the latter only in the oral and anal regions of the germ-band), and that vitellophags, or cells left in the yolk at a time when the remaining cleavage products are traveling to the surface to form the blastoderm, take no part whatsoever in the formation of the mesenteron, but degenerate *in situ* and finally undergo dissolution."

I have been unable to obtain a copy of Heymons's study of the germ-layer formation of Orthoptera and Dermaptera (14), but his conclusions have appeared in abstracts and are as follows: The yolk-cells take no part in the formation of the embryo. There is no true gastrulation process, but the under-layer arises from all parts of the embryonic area. When what is usually regarded as a typical gastrula invagination occurs, as in most insects, it is to be explained, not as gastrulation, but as a simple mechanical process caused by an aggregation of cells at one point. The layer generally known as the mesentoderm is in reality only mesoderm, the endoderm appearing relatively late and arising from the ectoderm of the stomodeal and proctodeal invaginations.

My results agree with Heymons's conclusions as to the origin of the mesoderm of insects primitively in a collection of cells arising diffusely from the ectoderm; but I must differ from him and agree with Wheeler in the latter's interpretation of the invaginate groove, from which the endoderm and mesoderm arise in most insects, as a true gastrula.

The Termite, which is certainly as primitive as any other insect hitherto described, exhibits no gastrula invagination. I have shown that the under-layer begins to appear at all points in the embryonic rudiment at an early stage of its formation. The plug of lower-layer cells, which becomes so prominent as the germ-disc grows more distinct, is apparently largely

the outcome of concentration of the cells of the disc toward the center. The relation of such a manner of formation of the under-layer to that generally described for insects is interesting to consider. This process does not appear to me to be derived from an invagination as a slurred gastrula. It is rather a method of delamination, where there is a further tendency in the lower-layer cells to collect toward a middle point.

A similar method has been described for Crustacea, Arachnids, and Myriopods, and all of these facts, taken together, lend weight to Heymons's contention that an indefinite migration below is the more primitive method of forming the under-layer in insects.

Heymons's explanation of the gastrula groove commonly found in insects, however, requires examination.

He does not attribute to such invaginations the significance of a process of gastrulation. From his standpoint the invaginate groove (which, as Wheeler points out, is so universally present among insects, and so essentially involved in the establishment of the under-layer) is a mechanical process and independent of the formation of mesoderm or endoderm.

I do not see the strength of this position.

In so far as this author finds the diffuse method of the origin of the mesoderm in certain Orthoptera the primary one, and offering a favorable basis for the origin of an invaginate gastrula, he seems justified.

I cannot, however, take the further step with him and dismiss the invaginate gastrula, found so universally among insects, as no gastrulation, but as simply a result of the crowding of an aggregation of cells at one point. Though we still have such an aggregation in the Termite, it has not in this group led to invagination as a mechanical necessity. In the place of invagination there is simply a crowding of certain ectoderm cells, arising at irregular points, below into a solid plug extending down into the yolk.

As far as our understanding of mechanical forces and their necessary results goes, the reason is not clear, without further addition, why the mesoderm came in other forms to arise in a groove instead of continuing to wander below in a solid mass.

That the under-layer is formed most easily and efficiently by a process of invagination seems evident, from the almost universal appearance of the gastrula groove in insects. Given first the more primitive, diffuse method of forming this layer still persisting in the Termite and, as Heymons claims, in other primitive insects, we may attribute to Natural Selection its improvement until an invaginated gastrula groove has become the common and readiest means of attaining the end. When we use Natural Selection as the agent of this change, we of course mean that the primary organic structure (in this case the mesodermal cell rudiment arising diffusely from all points of the ectoderm) was forced to respond to a further combination of forces in the environment which we cannot define in more exact physical terms.

From this point of view the usual method of forming the mesoderm in insects, by a well-marked gastrula groove, is not an independent or accidental phenomenon, but has been derived from a more primitive method of migration already established in the earlier insects, not as a direct and necessary result of apparent and readily stated mechanical conditions, but as a response to additional forces, compelling an important change in the older but less direct process which is still efficient in some primitive insects. These "additional forces" (mechanical, chemical, or what not), included under the general term "adaptive," did not "necessarily" disturb in the Termite the primitive habit established in their ancestors. In other insects, when new conditions (mechanical or others) made it possible and more desirable, invagination arose as a response.

A study of the origin of the "lower-layer" in the Termite shows a very close connection between this and the establishment of the first rudiment of the embryo by a concentration of the blastoderm cells toward a certain area (as in the case of Isopods discovered by McMurrich). This more general phenomenon must be first explained before attempting the special problem of the exact mechanical nature of the origin of the mesoderm, which is too intimately bound up with the solution of the former question to be considered alone.

As to the entoderm of the Termite, I must say that it

appears late, after the segmentation of the germ-band. The yolk-cells (as both Heymons and Wheeler claim) can take no part in the formation of this layer; since at an early stage, before the closure of the amniotic cavity, they have become very large and unlike the cells which later form the entoderm.

The fact that this layer arises so constantly among insects with the mesoderm at the two ends of the invagination, termed "gastrula" (see Wheeler (26)), is a strong point against Heymons's assumption of the independent, accidental character of this groove.

I shall be obliged to defer to another time the discussion of the method of the origin of the entoderm, its exact relation to the mesoderm and to the gastrula groove, when this occurs, as well as its association with the stomodeal and proctodeal invaginations.

THE ORIGIN OF THE AMNION IN INSECTS.

The discussion as to the cause of, and the primitive method of origin of, the embryonic membranes of insects has at least developed some extremely interesting ideas.

At present, opinions seem to halt between, first, the Ryder-Wheeler (26) hypothesis of a purely mechanical and independent origin of the amnio-serosal fold among the winged insects; and, second, the theory of Will (27), Wheeler (25), and Korschelt and Heider (17), recently championed by Heymons (15), which associates the formation of embryonic membranes in insects, more or less closely, with a certain phenomenon exhibited by the myriopod embryo. Wagner's (23) views I shall put, for convenience, in the first category; while Willey's (29) recent contribution, though in some respects agreeing with the second, will have to be considered alone.

A. Examining first the Ryder-Wheeler theory, we find that Wheeler (26) has adapted Ryder's (22) "mechanical explanation" for the origin of the amnion of vertebrates to the insect amnion. Of course the term "mechanical" is here used in its narrower sense, referring the question to immediate antecedent causes, which alone are claimed to necessitate the result. The

question whether the origin of organic structures is ultimately purely a problem of mechanics, as a first cause, is not raised. Here the contention is that certain evident and simply stated conditions of pressure and mechanical strain are alone sufficient to force the amnio-serosal fold to arise.

Wheeler (26) advocates this idea concisely, as follows: "The amnio-serosal fold is a mechanical result of a local induplication of the blastoderm, due to rapid proliferation in a single layer of cells." "There is the vesicular one-layered blastoderm filled with yolk, and the germ-band arising by rapid proliferation at one point. The resistance of the yolk being less than the external resistance of the tightly fitting chorion and vitelline membrane on the one hand, combined with the peripheral resistance of the extra-embryonal blastoderm on the other, the germ band is forced to invaginate. This invagination is favored by the displacement of yolk during its liquefaction and absorption by the growing embryo. We may suppose that this invagination, which results in the formation of the amnio-serosal fold, assumed a definite and specific character in different groups of insects."

Similar mechanical conditions are appealed to as the cause of certain invaginations in other forms; the Cestode head in *Cysticercus*; the Nemertine in the *Pilidium*; the formation of the young *Spatangid* in the *Pluteus*; the development of the amnion and serosa in vertebrates; and the imaginal discs of insects.

a. 1. Even if we admit the presence of just such a combination of forces as is enumerated above, they seem to be subsidiary and insufficient alone, without a further cause, to explain the origin of the membranes for the following general reasons:

It must be recalled that no amnion results in the similar rapidly proliferating areas of crustacean eggs, that such a membrane is lacking among the myriopods and apterygote insects (in spite of Heymons's (15) claim, which requires further and more convincing proof, as we shall see later), and that it is not formed in certain of the higher insects. It should also be remembered that similar membranes are want-

ing in anamniote vertebrates, where the mechanical conditions, as far as this theory goes, appear to be much the same as in amniote vertebrates.

Apparently similar conditions of pressure and mechanical strains would be brought to bear on the embryonic areas of the myriopods, the apterygota, or crustacea, as are claimed to necessarily force the formation of the amnion of insects, but no amnion appears in the former groups. The invaginations which do occur (to form the eyes, the digestive tract, etc.) in some of the rapidly proliferating areas of the decapod blastoderm would be generally thought to necessitate something more than such an enumeration of mechanical strains to explain them.

In those highly specialized insects that entirely lack an amnion, its failure to appear is even more marked. Here, within the same group, there are forms which, in the face of the forces above stated as sufficient to produce an amnion, have none.

The effort to apply such a simple mechanical explanation to the origin of various organic larval structures may seem plausible at first sight; but, carried to its logical limit, not so much so. Why stop at the structures mentioned? Might not the germ-layers, the central nervous-system, as well as other such rapidly proliferating areas, be as readily included? Heymons, as I have shown, has already attempted an affirmative answer for the origin of the gastrula groove.

a. 2. Turning from such general considerations to my own special results, the formation of the caudal flexure of the *Termite* seems a case in point.

This ventral flexure of the tail end of the embryo, as I have pointed out, at first seems just as reasonably to be ascribed, solely and directly, to a necessary result of pressure or mechanical strain as the instances referred to by Wheeler. A single unusual specimen proved beyond doubt such a conclusion to be false, and that what might appear superficially to be a necessary method of growth could be accomplished in an entirely different manner. It was certainly proved to be independent of the resistance of the chorion, which seemed so determinative at

first sight. Here was another case of the nearest explanation not necessarily being the true one.

It seems hardly necessary to say that the fact that such invaginations can be watched step by step sometimes, and can be actually observed to encounter resistance at every stage, is no proof that such resistance causes the process.

a. 3. My study of the formation of the embryonic rudiment and of the origin of the amniotic fold of the Termite indicates forces of a very different nature from those formulated by Wheeler; in fact the very reverse.

As the germ-disc becomes sharply defined, the area of the blastoderm occupied by it is distinguished by the closer crowding of its cells, while the surrounding cells become flattened and pulled apart into a thin membrane. There appears to be a contraction toward the embryonic area, as is observed in the formation of the embryonic rudiment in other insects and other arthropods. At any rate, the extra-embryonal blastoderm may be said to be stretched and kept so by the changes taking place in the embryonic area.

Before the amnion arises it is clearly differentiated as a special thickened area of the germ-disc. When the embryonic rudiment doubles-up, and this posterior portion of it folds over to become the amnion, the extra-embryonal blastoderm is pulled forward and further stretched.

It seems correct to speak of the tension of the serosa as due to the activities in the embryonic area, rather than to reverse the case and explain important changes in this area as a result of such tension.

In studying the growth of the germ-disc, I can find no indications of a rigid resistance to its growing edges claimed to be offered by the rest of the blastoderm. The cells around the rapidly proliferating area do not seem to be fixed, immovable points; and the membrane they form does not appear to be more resistant to this more active area than is the yolk.

Another important point is the fact, as I have shown, that the amnion is not a derivative of the extra-embryonal blastoderm, as Wheeler (26) concludes in his latest paper.

If the Ryder-Wheeler mechanical theory were correct, the most natural place to expect the fold would be just at the junction of the rapidly proliferating germ-disc with what is claimed as a rigid, resistant, extra-embryonal region. We would look for the weakest point here. The fold does not, however, occur here in the Termite or other amniote insects.

My own observations, and a general review of the question, lead me to believe that the embryonic membranes of insects are adaptive structures, which arose in the winged insects as a response to some definite need of the embryo. I do not think the exact combination of physico-chemical forces, coöperating to bring about this result, can be stated at present. The eggs of the anamniote apterygota are, to all appearances, as far as mechanical conditions go, similar to those of winged insects. The physical constitution of the egg was already favorable to the origin of the amnion in the ancestors of the latter forms; but before one arose, certain additional forces were necessary, which must be associated with some necessity of covering over the embryo at an early stage. Whether this necessity (physico-chemical, no doubt) was one of protection, prevention of evaporation, better nutrition, or to furnish a depository for waste products, may not be decided; but any one of these suggestions, or all together, would be reasonable cause.

When forms arose among the higher insects, as adaptations to special new conditions, the early completion of the process became less important; and in a few cases the amnion ceased to appear, being no longer needed. (If it is any more precise, we may say that the amnion was no longer maintained by the physico-chemical forces which originated it.)

B. I must refer to Wagner's (23) comprehensive theory of the origin of insect embryonic membranes and other organic structures, as another example of a simple, clear-cut mechanical explanation of such problems, which also illustrates the difficulty of correctly estimating and balancing forces, and their necessary effect on organized matter.

In a few words, his idea is as follows: Think of the similar cells of a uniform epithelium as an organic molecule, so built

up together that a certain homogeneous reciprocal relation is attained. Now, when certain cells of this layer become altered in nature under the influence of some special forces, the reciprocation with neighboring cells is likewise altered, resulting in so changing the relations with these latter that a separation of the changed cells from the layer of similar, unchanged cells must take place.

The mechanical basis of the theory is what happens when a foreign, inorganic particle is introduced into a fluid or viscid layer ("Haut") whose elemental drops coöperate reciprocally to form a uniform sheet. The foreign particle would be thrown out as a result of purely mechanical tensions.

So in the case of the origin of the germ-layers, by invagination or immigration; the sub-epithelial muscle cells of Medusae; gland cells; central nervous-system; sense organs; Cestode head in the cysticercus; the extra-embryonal and embryonic cells; embryonic membranes; imaginal discs, etc. Whenever two kinds of cells occur in an epithelial layer, one sort is thrown out, so to speak, by invagination or immigration. Cases where this has not taken place represent the early stages of the process (as certain epithelial gland cells or muscle cells). In all these cases the common and necessary cause of invagination and immigration is claimed to be the sharp differentiation of certain cells, physically and chemically, to such an extent that they must move from their primitive position.

It seems hardly necessary to observe that, though this theory is strictly logical and far-reaching, Wagner does not explain the fundamental question why certain cells rather than all are modified; and that he overlooks an important and essential difference between the living modified cell in the uniform cell layer, on the one hand, and the foreign, inorganic, dead particle in the homogeneous fluid layer on the other. The theory must collapse when we reflect that, instead of being obliged by the supposed necessity to immigrate as an inorganic particle, the modified living cell could accommodate itself to its old neighbors and remain with them. Of course the inorganic particle would have no such power of adaptation resident in living protoplasm. Possibly this adaptability of living substance

is the reason Wagner finds gland cells, muscle cells, etc., not wandering out of the otherwise uniform layer. When a migration of specialized cells does take place, we shall have to look further than to such a simple statement of inorganic physics for the explanation.

C. I shall not examine Willey's (29) hypothesis at length, since, in as far as it refers to the origin of the amnion, it appears to be largely a statement of Heymons's (15) views, which will be considered further over.

Willey's main thesis seeks to prove, by reversing a theory of Hubrechts's, that the extra-embryonal blastoderm of the insect egg (*i.e.*, the serosa and amnion) is a secondary cellular membrane, derived in a curious roundabout manner from a more primitive, extra-embryonal trophic membrane, "the trophoblast"; which, "as it is preserved to us in the embryo of *Peripatus novae-britanniae*, arose in adaptation to a viviparous habit acquired by the terrestrial descendant of an aquatic ancestor; and that it became transformed, whether directly or by substitution, into the serosa, in correlation with the secondary deposition of yolk-laden eggs."

The following fundamental assumptions seem to me inadmissible: That the viviparity of *Peripatus* is primitive; that "lecithality and deposition of the eggs of insects are both secondary"; that this application of the idea of substitution involving the reverse of Hubrechts's idea is reasonable; that the amnion is a derivative of the extra-embryonal blastoderm in insects; or that the serosa of the insect egg has any such indirect phylogenetic history, believing it as I do to be directly comparable to the inactive extra-embryonal surface cells (Deckschicht) of other yolk-laden eggs.

D. 1. I have already discussed, in the division of this paper headed General Conclusions as to this Type of Insect Development (page 29), certain aspects of the theory presented in Korschelt and Heider's text-book. Reference must be again made to that section of my paper, where the original sources and criticisms of the theory are quoted. This theory, which originated with Will and Wheeler (25), was later modified by Heider.

The origin of the embryonic membranes of insects is referred to the peculiar phenomenon of "doubling-up" exhibited by the myriopod embryonic band.

It was originally claimed by Will that the invaginations in the two cases are genetically connected to such an extent that some of the posterior segments of the elongated myriopod band, on bending forward, were directly transformed into the amnion in the ancestral insect embryo. In this way the adult insect came to have fewer posterior abdominal segments than the myriopod.

Judging from Heider's remarks in his monograph on *Hydrophilus*, no genetic relationship is now meant to be implied between the "doubling-up" of the myriopod embryo, and the invagination to form an amnion in the insects.

The idea now advanced is, that the resemblance in the two cases is of sufficient importance to intimately connect the phenomenon presented by the elongating myriopod embryo with that observed in Libellulids, which are said to best exhibit what is termed the primitive, invaginate type. The similarity in the two instances, however, is only claimed to be the result of the action of a common cause of the invagination.

Will's idea of a transformation of a part of the segmented body of the embryo of the myriopod into an amnion for the insect embryo has been abandoned since Graber's (9) criticism.

Heider in his monograph (13), and in the text-book with Korschelt (17), points out the similarity between the amnion and the rudimentary ectoderm of the embryonic band from which it arises; and in this is in agreement with what has been observed and figured by most investigators, as I have already noted in another connection.

D. 2. Recently, Heymons (15) has studied the interesting apterygote *Lepisma saccharina*. As a result, he claims to have furnished us with a convincing intermediate stage between the phenomenon of doubling-up of the myriopod embryo, and the formation of an amnion and amniotic cavity in winged insects; which he thinks proves that the latter process is directly derived from the former.

In describing the embryonic rudiment the author speaks

of the entire extra-embryonal region as a serosa, before the doubling-up takes place. As this happens, he says (in a paragraph on page 587 of his paper) that the cells of the edges of the embryonic band become pulled out into a thin cellular membrane, the amnion. None of his figures, however, give proof of such a process of transformation of a part of the

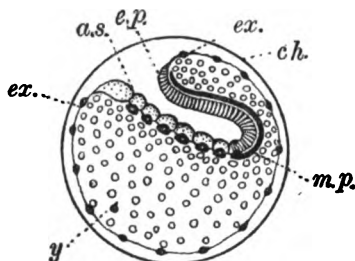


FIG. 1.

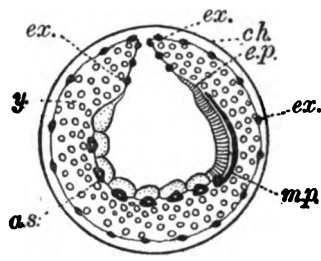


FIG. 2.

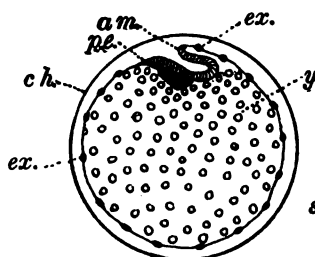


FIG. 3.

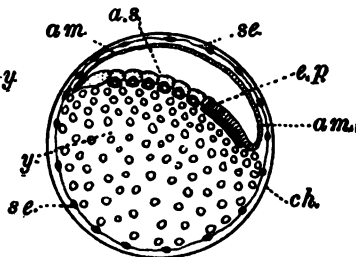


FIG. 4.

Diagrammatic figures (sagittal sections), comparing the "primary ventral flexure" ("doubling-up," or invagination) of the myriopod and apterygote embryos with the amniotic fold of the winged insect. (1) Myriopod (*Julus*) embryo, at the first appearance of the ventral flexure; (2) similar stage of the embryo of *Lepisma* (apterygote); (3) early amniotic fold (doubling-up, or invagination) of the unspecialized embryonic rudiment of the Termite; (4) later stage of the Termite embryo, after the closure of the amniotic cavity—a stage similar to that of the two types in 1 and 2, but the "tail-piece" is straight here. *ex.* extra-embryonal blastoderm; *e.p.* posterior, unspecialized ectoderm; *m.p.* posterior, unspecialized mesoderm; *a.s.* segmented region anterior to unspecialized tissues of "tail-piece"; *ch.* chorion; *y.* yolk; in 3, *p.e.* primitive, unspecialized ectoderm of germ-disc; *am.* amniotic fold in undifferentiated ectoderm; in 4, *se.* serosa; *am_i.* amnion.

embryonic, rudimentary ectoderm into the amnion, as observed in the winged insects. The figures show no more as to this question than that, as the embryonic band sank into the yolk, the extra-embryonal cellular membrane attached to its edges was pulled along.

The result, as figured, is a band doubled-up like that of the myriopods, and not differing from this except in lying deeper in

the yolk, into which some of the extra-embryonal membrane is dragged down. (See my Text-Figure 2.)

Comparing the diagrammatic text-figures here copied, — one, Fig. 1, after Heathcote (31), Fig. 14, showing the doubling-up of the myriopod (*Julus*) embryo, and another, Fig. 2, after Heymons (15), for *Lepisma*, — we find the thick germ-band in each case enclosing a cavity. In the myriopod egg the two ends of the embryo are on the surface, and there pass into the thin extra-embryonal membrane. In the *Lepisma* egg the embryo lies more internally. Except for this, there is no essential difference apparent in the relations of the extra-embryonal blastoderm to the embryo or in the nature of the open cavity. Compare these two figures with the third text-figure of the Termite, at a stage before the closure of the amniotic cavity, and with the fourth figure of the closed amniotic cavity of the Termite. (All eggs are represented as spherical for better comparison.)

Does the so-called amniotic cavity of *Lepisma* constitute any nearer approach to the true amniotic cavity than the one previously found between the “doubled-up” body of the myriopod embryo? I think not, without a further and more convincing series of figures of some stages in the formation of the so-called amnion, which would prove it to be any more truly an amnion than the part of the blastoderm external to the embryonic band of the myriopod, or in any way different from this, except in being pulled down into the yolk.

Instead of being an important intermediate stage between the phenomenon exhibited by the myriopod embryo and the formation of a true amnion, and amniotic cavity, in the winged insects, there is nothing in the figures (nor does the single descriptive paragraph convince without further figures) to give reasonable grounds for the claim that there is any such difference between the phenomenon exhibited by the myriopod and that shown by Heymons for *Lepisma*.

The gap between the open cavity in the doubled-up myriopod embryo and the true, closed amniotic cavity of winged insects, seems just as wide as before *Lepisma* was studied from this standpoint; except in as far as the apterygota have been

shown to exhibit this phenomenon similar to the myriopods — an important point in itself, indeed, if the amniotic fold of the winged insect is to be derived from an earlier invagination.

Willey differs with Heymons as to interpreting the ventral flexure of the embryo of *Lepisma* as comparable with the later caudal flexure of insect embryos. Without admitting his theory of the trophoblast, I must agree with Willey in this distinction.

In many respects the development of *Lepisma* bears a close resemblance to that of the primitive Orthoptera and the Termite. It is interesting to find the germ-disc originating at the posterior pole of the egg as in the Termite. The absence of a gastrula groove, in connection with the origin of the mesoderm, is also in agreement with what Heymons has found in some Orthoptera, and with the results here submitted for the Termite.

E. The conclusions reached from the above general review of the question before us, in the light of my own special observations, and again referring to my views, expressed in a previous section of this paper, as to the primitive type of insect development, may be summed up as follows :

e. 1. The amniotic fold did not arise as a necessary result of any combination of purely mechanical forces which has been formulated up to the present time.

e. 2. The amnion and amniotic cavity of insects are adaptive structures, which, as far as our knowledge now goes, arose first in the winged insects as a response to some definite need of the developing embryo.

e. 3. The amnion is primitively a derivative of the rudimentary embryonic ectoderm.

e. 4. An "invaginate" type of development is the more primitive one for insects. Irrespective of its relation to the phenomenon of doubling-up of the myriopod or apterygote embryo, it has been shown to be associated with the more primitive insects, and the most primitive (probably) method of membrane formation outlined in paragraph 5 below.

It must be added to this, that in the light of researches of a more recent date than that of the publication of the text-book of

Korschelt and Heider, it is found that the Termite and certain Orthoptera with superficial embryos, as is explained in this paper, represent the invaginate type of development there suggested better than do the Libellulids, with embryos "immersed" in the yolk and other secondary characters. Other methods of origin of the amniotic fold are most probably derived from that best exhibited by the Termite and certain Orthoptera.

e. 5. It became important for some reason (whether for protection, better nutrition, accumulation of waste products, etc.) associated with a new habitat or mode of life, that the superficial embryos of the ancestors of the winged insects should be completely covered over. The forms we may now consider primitive for a number of reasons exhibit a relatively small, superficial disc as the first rudiment of the embryo. Here was an especially favorable condition for the earliest possible appearance of the membranes, at a time when they might be particularly needed. Only a few forms have retained this process in a near approach to its primitive form. (I believe that the amnion is formed from the rudimentary ectoderm by essentially the same method, on the similar germ-discs of the Termite and certain Orthoptera, though in the Termite the fold is more evident at the posterior end.) Changed conditions have led to a disappearance of the membranes in a few insects.

e. 6. The ventral flexure of the first rudiment of the embryos of the invaginate type which forms the amniotic fold has not been proved to be of a phylogenetic significance.

e. 7. Even if it can be shown conclusively, in the case of the apterygote egg, that the open cavity is a somewhat nearer approach to the amniotic cavity of the winged insect than that found in the myriopod egg (or, in other words, what Heymons speaks of as amnion is a derivative of the rudimentary ectoderm, as in the Termite, and not simply a part of the blastoderm comparable to that lying outside the limits of the embryo in the myriopod egg), it must be remembered that the open invaginations of the myriopod and apterygota may not even be due to causes similar to those calling for the closed amniotic cavity of winged insects. These may be entirely distinct phenomena with very different significance.

There is, however, undoubtedly a resemblance between this invagination and the phenomenon exhibited by the myriopod embryo, which is strengthened by the appearance of the same condition in the apterygote egg. This suggests strongly a common cause (the general adaptive nature of which I have suggested in agreement with others already quoted); but Korschelt and Heider's ((17), pp. 734 and 787) further idea, that this cause is associated necessarily with the resistance offered by the spherical chorion to the growth of the elongating germ-band, does not seem convincing, since these authors themselves suggest an objection in the different behavior of some myriopods, see (17), p. 735; since similar conditions do not necessitate a like invagination in certain insects, or in the elongating band of the arachnid or in that of a fish, for reasons I have suggested in another place; and since the amniotic fold of the Termite arises on the nearly circular disc, before such conditions would be effective.

e. 8. The possibility of a connection with the invagination ("doubling-up") of the myriopod embryo seems sufficiently strong to warrant a new statement of how a fundamentally similar invagination, in the primary embryonic rudiment of the myriopod-like ancestors of winged insects, may have formed a starting point for the formation of an amnion.

As has been pointed out, some theory associating the two invaginations has seemed probable to a number of investigators.

Will (27), basing his theory on a study of the Hemipteran embryo, first insisted on a derivation of the amnion from a region of the myriopod body.

Wheeler (25), at about the same time and independently of Will, advocated much the same idea, though he simply quotes Will in regard to the degeneration of segments into an amnion.

Graber (9) justly criticised the idea of a disappearance of certain posterior abdominal segments of the myriopod-like ancestors of the insects, by a degeneration into an amnion and a forward migration of the anus.

Finally Heider (13), and later Korschelt and Heider (17), presented a modification of the theory I have outlined, which,

accepting a fundamental common cause connecting the two invaginations, abandoned further comparison.

Heymons (15) has quite recently claimed to have carried this a step further, in a manner which I have already considered.

If we proceed from the assumption that some like necessity of removing the embryo from surface insults, or of furnishing it with better conditions of nutrition, etc., caused an invagination of the embryo of the myriopod (or apterygote) and of the superficial rudiment of the ancestral winged insect, it is possible in the case of the Termite embryos at an early stage, just before the closure of the amniotic cavity, to make a comparison of a somewhat different nature from what has hitherto been suggested.

The condition found in the Termite permits us to see how we may retain an essential feature of Will's idea (also Wheeler's) of a derivation of the amnion from a portion of the ancestral myriopod's embryonic tissue, in association with a process of invagination, without involving the further idea of a transformation of definitively organized tissue, with the disappearance of segments and the migration of the anus. It will, however, be found that the following is not an effort to trace the amnion in a phylogenetic sense back to the myriopod.

Referring back to the text-figures, we find practically the same condition in the three first diagrams—a doubled-up, comparatively thick embryonic band, enclosing a cavity which opens on the surface of the egg. In the Termite this opening in Text-fig. 3 closes, and the outer wall of the cavity, which is a portion of the ectoderm of the first rudiment of the embryo, becomes the amnion. (See fourth text-figure.)

It is evident that if the invagination of the winged-insect embryo is to be derived from that in the myriopod (or apterygote) egg, the amnion of the insect most probably arose from some portion of the thickened, unspecialized (striped in the text-figures) ectoderm of the myriopod (or apterygote) ancestor.

My idea is that, since it has been shown that the amniotic fold of the Termite is a specialized portion of particularly the posterior ectoderm of the embryonic rudiment, at a very early stage, before elongation begins and before the appearance of

segments or the anus, the comparison with the embryonic, invaginating rudiment of the apterygota and myriopod should be made with the ectoderm alone, and at a correspondingly early stage in its differentiation.

My effort is not to derive the amnion from a portion of the myriopod body in a phylogenetic sense; but to explain how, in association with a fold similar to that of the myriopod-like ancestor, but appearing sooner, it may represent an early specialization of the undifferentiated tissue (ectoderm) of a primary embryonic rudiment common to the two arthropods (see B, pp. 30-31); and how this folding off of the amnion need not prevent the usual continuation of the development into an elongated embryo comparable to the myriopod.

Such a comparison may be readily made by referring back to the diagrammatic text-figures.

These figures of course represent actual stages in the development of the three forms. The two upper figures illustrate the first appearance of the ventral flexure (doubling-up) of a myriopod, Text-fig. 1 (Julus, after Heathcote), and of a wingless insect (the apterygote *Lepisma*, after Heymons), Text-fig. 2.

Following Heathcote (31) in his description of the myriopod development, we find in the first text-figure that the ventral flexure occurs here comparatively late in the ontogeny, after a few anterior segments have been formed from both layers. The important fact to note is that the bending takes place just behind the last segment differentiated, and in a region that Heathcote speaks of as unspecialized tissue, commonly termed the "tail-piece." I have indicated in the diagrams the usual sharp distinction between the early ectoderm and mesoderm in this posterior region, Text-fig. 1 (see Heathcote's Fig. 30). (Note that the as yet undifferentiated ectoderm is striped in the diagram, while the similar mesoderm is a simple black line.)

Examining Heymons's results for *Lepisma*, as represented in the second text-figure (Text-fig. 2) to the right, we find essentially the same conditions as in the myriopod. (See his Fig. 1, (15), for the sharp separation of primary ectoderm from mesoderm.)

Turning finally to the two lower text-figures of the developing Termite embryo, we recall that the germ-disc, when the amnion first folds up and before the closure of the amniotic cavity, is in a very undifferentiated state. (The suggestion is made in a previous section of this paper (*B*, p. 30) as to the comparatively primitive nature of this small rudiment.) I have indicated in the diagram illustrating this stage (Text-fig. 3) that the entire rudiment, ectoderm and mesoderm, is quite unspecialized. The upper layer is striped, as is the ectodermic tissue of a similar early stage in differentiation in the "tail-piece" of the other figures. The lower layer is also an unspecialized mass.

The condition of the tissues is just what was found in a much later stage of the myriopod (or apterygote), in the particular region where the ventral flexure occurs (Text-figs. 1 and 2).

A first difference is, that though the flexure takes place at a corresponding stage in the differentiation of the tissues, it occurs at a much earlier period in the development of the winged insect; in fact, at what I have pointed out is the earliest possible stage for the origin of an amnion. Another and second point is that only the ectoderm is here concerned in the flexure. Thirdly, if a posterior portion of the primary unspecialized ectoderm becomes amnion, what will be the effect on the further development of the embryo?

The first point of difference, the relatively very early appearance of the flexure in case of the insect, may be unimportant; since the two invaginations before us develop at a like stage in the differentiation of corresponding tissue (see text-figures). We have suggested apparently good reasons for an especially early folding, or invagination, of the superficial rudiment of the ancestral winged insect. Text-fig. 3 shows this taking place before any segments have been differentiated.

As to the second point, in regard to the ectoderm, it must be first recalled that Heathcote's Fig. 30 for *Julus* indicates a special participation of the ectoderm in the flexure, when first beginning. Further, in both the myriopod and the apterygote on the one hand, and the winged insect on the other, there is a marked separate though associated development in the upper

(ectodermic) and lower (mesodermic) layers when once established. Each layer develops certain structures peculiar to it (text-figures). The primitive ectoderm alone would, on *a priori* grounds, be expected to be the layer to differentiate a protective structure, as the amnion has been thought to be. Finally, in the Termite the doubling-up to form the ectodermic amniotic fold takes place distinctly before the mesoderm has spread beneath the posterior region, where the process is inaugurated (Text-fig. 3).

The third point suggested was the effect on further development of the early formation of an amnion from the unspecialized posterior ectoderm. It is interesting to observe, as the fourth diagram (as well as the final plate in this paper) shows, that after the formation of the amnion as one of its organs, the ectoderm, as well as the mesoderm beneath, continues to grow posteriorly, carrying the amnion behind and budding anteriorly the ectodermic portions of the segments of the body until, finally, we reach a stage identical with that of the myriopod or apterygote. The unspecialized tail-piece of this stage was formed in the usual manner from the original, undifferentiated, posterior tissue of the primary rudiment, from which the amnion arose at an earlier stage.

In a sense the formation of the ectoderm of the tail-piece, in this later stage of the winged-insect embryo, may be thought of as a regeneration of the lost terminal material which went into the amnion; just as a piece of the ectoderm of a developing hydra (worm, or other form) might be removed at an early stage, without disturbing the further development of parts from the ectoderm, since the ectoderm remaining would supply the loss. This statement must, however, be accepted as an illustration of regeneration from undifferentiated tissue, only in so far as such a process is comparable to normal growth following the differentiation of an organ from unspecialized tissue.

From this point of view the amnion is not a substitute for, or a transformation of a posterior region of the myriopod body. It is not derived from any previous structure. It is a specialized structure folded off in the winged-insect embryo, for some adaptive reason similar to those causing the doubling-up of the

myriopod embryo, at an especially early period, from the primitive unspecialized ectoderm of an ancestral disc-shaped rudiment. It arises especially in a posterior region of the early ectoderm of the Termite. A similar primitive origin may, however, be associated with the sinking of the embryonic rudiments of other insects (especially in the forms I have taken as primitive), where the folding occurs in other portions of the early ectoderm, for it must be remembered that such ectoderm can produce lateral as well as serial organs.

The less number of segments in the winged-insect or apterygote, as compared with the myriopod, was attained, as far as the embryology shows, by an arrest in a primitive method of growth common to arthropods and similar to budding, which was continued for a longer time in the many segmented ancestors of insects.

The reason for the shorter duration of this process in the later group is not known, but must be sought in such related fundamental problems of growth as regeneration of lost parts, metamerism, and the cleavage of the ovum.

e. 9. As has been said, the ventral flexure of myriopod embryos of the present time may be proved later to be connected in no sense with an amniotic fold. Even if such turns out to be the case, the above comparison will then have served a good purpose, in calling attention to a plausible interpretation of the amniotic fold as originating primarily by invagination in winged-insects, independently, and not traceable to any previous similar phenomenon.

e. 10. If the above view is applied to the vertebrate amnion, the participation of both primary and, at the point of origin of the fold, undifferentiated layers of the body-wall would be understood in a sense similar to the formation of other early organs, in which both primary layers coöperate.

EXPLANATORY NOTE.

This paper was accepted as a thesis, May, 1896. It was abstracted in the *Johns Hopkins University Circulars*, Vol. XV, June, 1896. Unavoidable delay in publishing and a renewed

study of some additional and better material have rendered the present revision advisable. I have hence included a consideration of two recent papers, that of Heymons (15) and that of Willey (29). It is a pleasure to here thank Professor C. O. Whitman for many courtesies extended to me during two summers' work at the Woods Holl Marine Biological Laboratory.

AUGUST 16, 1899.

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EXPLANATION OF FIGURES.

All figures drawn with aid of camera. From Figs. 1-29 inclusive, about the same magnification. Figs. 1-24 drawn with Zeiss *oc.* 4. *Object. A.* magnifying 97 times. Figs. 24-29 inclusive, drawn with Bausch & Lomb's tube 160 mm., *oc.* 25 mm., *object.* 17 mm., magnifying 96 times. Figs. 30-37 inclusive, drawn to same scale with Bausch & Lomb's *oc.* 25 mm., *object.* 4.2 mm., magnifying 450 times. Tube 160 mm.

EXPLANATION OF PLATE XXIX.

(The posterior ends of all eggs are placed uppermost.)

FIG. 1. Ventral surface of egg tipped up somewhat to show micropyles at posterior end. *A.*, anterior; *P.*, posterior; 1-9 on each side the micropylar funnels.

FIG. 2. Optical section of egg with one nucleus, segmentation nucleus, in center. Egg stained in borax carmine and viewed as a transparent object in clove oil. Yolk bodies not shown in figure. The chromatin of the nucleus is seen in the center of a small mass of lightly stained protoplasm: *p.b.*, polar bodies.

FIG. 3. Optical section of egg with two nuclei: *p.b.*, polar bodies; *d.n.*, dividing nucleus, in which the chromatin is separated into two masses.

FIG. 4. Optical section of egg with four nuclei. The nuclei are not all in the same plane. A line connecting the two posterior nuclei is in a plane at right angles to one joining the two anterior nuclei lying in the plane of the paper. To reach this position, the axes of the spindles of the two dividing nuclei of the last stage must have rotated in opposite directions. See McMurich on Isopods for a similar phenomenon (18). All the nuclei are dividing, *d.n.* The chromatin of the polar bodies has become much fragmented, *p.b.*

FIG. 5. Optical section of egg with nine nuclei. Nuclei scattered in yolk. Axes of dividing nuclei of last stage have rotated, as before, to make angles with one another. An odd nucleus shows irregularity in divisions: *p.b.*, polar bodies.

(All the remaining figures of this and the next plate are surface views.)

FIG. 6. Ventral surface of older egg. The cells are at equal distances apart.

FIG. 7. Surface view of right side of egg with more nuclei than the last. The nuclei somewhat more numerous in the posterior half: *p.b.*, polar bodies.

FIG. 8. Ventral surface. Nuclei dividing, *d.n.*, everywhere on surface. Numerous pairs of just separated nuclei, *s.n.*, show division anteriorly as well as posteriorly.

FIG. 9. Ventral surface of egg with twice the nuclei of last. More cells in posterior half, due to movement that way and to multiplication.

FIGS. 10 and 10^a. Ventral and dorsal surfaces of egg with double the nuclei of last stage. The nuclei of the posterior half of the egg are more numerous than on the other end. They lie rather close together, nearly as far forward as the smaller diameter.

FIGS. 11, 11^a, and 11^b. Ventral, dorsal, and lateral surfaces of an older egg. In Fig. 11 of ventral surface, note that the anterior limit of the area *ca.* of relatively closely crowded nuclei of last stage has drawn nearer the posterior pole, away from the smaller diameter of the egg.

EXPLANATION OF PLATE XXX.

FIG. 11^a. Dorsal surface. The cells at posterior end of this surface crowded closely together to form posterior border, *p.b.d.* (posterior limit of disc) of the area *ca.* of ventral surface. The collection of nuclei forming this border are in sharp contrast to those scattered over this surface.

FIG. 11^b. Side view, giving better idea of how markedly the cells on the surfaces of the egg have crowded back to the region marked *ca.* in figures: *p.l.d.*, posterior limit of cap or disc well shown; *a.l.d.* anterior limit of disc; *A.* and *P.*, anterior and posterior.

FIG. 12. Ventral surface of older egg with disc of nuclei forming area *ca.* about equally distributed to its borders.

FIG. 13. Further contraction of disc; all of its boundaries now well within limits of ventral surface: *l.b.d.*, lateral border of disc.

FIG. 14. Ventral surface of last, seen slightly on one side to show outlines of contracting area.

FIG. 15. Posterior end of egg tipped up to show an especially marked concentration of embryonic disc.

FIG. 16. Ventral surface of older germ-disc torn off with a piece of the chorion: *ch.*, chorion; *ul.p.*, under-layer plug.

FIG. 17. Ventral view of egg showing germ-disc with crowded posterior margin: *am.t.*, amnion thickening, later to fold forward; *ul.p.*, under-layer plug more distinct.

FIG. 18. Ventral surface showing *am.t.*, amnion thickening, at its maximum of crowding before folding over.

FIG. 19. Older egg, somewhat on side to show amnion fold, *am.* The *ul.p.*, extensive under-layer plug. Cells of serosa, and middle thin region of disc, faint.

FIG. 19^a. Same stage dissected off to show details. Amnion fold reaches forward on sides anterior to *ul.p.* Anterior limit of disc, *a.l.d.* Note dividing nuclei, straight black rods.

FIG. 20. Side view of amnio-serosal fold, *am.*, soon after its origin. Cells of embryo not shown: *sc.*, serosa cells posterior to fold; *em.d.*, embryonic disc; *sc.a.*, serosa cells on yolk anterior to embryo; *ch.*, chorion.

FIG. 21. Side view of amniotic fold (partly in optical section) half covering the disc: *am.*, amnion, of same appearance as ectoderm of disc, being several layers thick; *am.s.*, side view of amnion; *my.*, two micropyles in distended, wrinkled chorion, *ch.*

FIG. 22. Optical section from side of closing amnion; *o.amc.*, opening from exterior into amniotic cavity; *sc.*, serosal cells, large and faint on surface of yolk; *yc.*, yolk-cells deeply stained and lying within the yolk. Cells of embryo and amnion not shown.

FIG. 23. Same stage as foregoing seen from surface. Lettering as before.

EXPLANATION OF PLATE XXXI.

FIG. 24. Ventral surface of egg, just after closure of amniotic cavity: *a.e.*, anterior end of embryo; *yc.*, yolk-cells intensely stained; *sc.*, serosal cells, large and of light color.

FIG. 25. Side view (optical section) of slightly older embryo, slipped back out of its usual position. The black ectoderm appears thicker in such an optical section than it actually is: *y.*, surface of yolk-mass; *a.e.*, anterior end of embryo; *ch.*, chorion; *am.*, amnion; *ul. (mes.)* under-layer, or mesoderm, extending forward beneath ectoderm.

FIG. 26. Ventral view of germ-band two or three stages older than Fig. 24, unsegmented and without cephalic lobes. The germ-band at this age is usually placed as in the next figure.

FIG. 27. Embryo like that in the last figure, in its usual position. Seen from side: *a.e.*, anterior end; *ch.*, chorion; *am.*, amnion; *p.e.*, posterior end.

FIG. 28. Unsegmented germ-band with cephalic lobes, just before the appearance of segments. Same stage as that of next figure, Fig. 29. The embryo is dissected off from the yolk, and drawn with lower (yolk) side uppermost: *am.*, amnion cells, along edges of band; *a.t.*, anterior triangular area, between cephalic lobes. The mesoderm cells are large black masses.

FIG. 29. Unsegmented germ-band in same stage as last. Side view, to show position on yolk. The amnion is faintly seen as a row of small dots beneath the chorion.

FIG. 30. Cross-section through middle of germ-disc, at about the age of Fig. 13, perhaps slightly older: *ul.n. (mes.)* under-layer or mesoderm nucleus crowded below the surface; *d.n.*, nucleus dividing to separate a cell below; *yc.*, yolk cells; *ybs.*, yolk bodies of all sizes, and two perforated with holes left by solution of oil drops. (0.003 mm. thick.)

FIG. 31. Cross-section through region of under-layer plug, *ul.p. (mes.)*, the mesodermal rudiment, at the stage of the germ-disc shown in Fig. 18; *d.n.*, nucleus dividing to separate cell below; *my.i.*, inner opening of micropyle; *my.c.*, penetrating canal of micropyle through chorion; *my.o.*, outer opening of micropylar funnel; *ch.*, chorion; *p.yb.*, perforated yolk bodies. A large yolk-cell lies under the middle of section. Yolk bodies are large. (0.003 mm. thick.)

The following six sections form a series, illustrating the growth of the germ-disc and the mesoderm; also the origin and growth of the amniotic fold. Nuclei diagrammatic, except the dividing ones.

FIG. 32. Median sagittal section of disc at stage in Fig. 18: *am.t.*, amniotic thickening of ectoderm; *ul.p. (mes.)*, under-layer or mesodermal plug; *a.e.*, anterior end of ectoderm of disc; *yc.*, yolk-cells; *yb.*, yolk body. Four dividing nuclei. (0.004 mm. thick.)

FIG. 33. Median sagittal section of disc, Figs. 19 or 19^a; *sc.*, serosal cells; *am.f.*, amniotic fold; *ul.p. (mes.)*, under-layer or mesodermal plug; *a.ec.*, anterior ectoderm; *yc.*, yolk-cell. Seven nuclei in various stages of division. (0.004 mm. thick.)

FIG. 34. Median sagittal section of an embryo slightly younger than that in Fig. 22: *sc.*, serosal cells; *y.*, yolk-mass of finely fragmented bodies beneath embryo; *am.f.*, amniotic fold; *o.amc.*, opening into amniotic cavity; *a.ec.*, anterior ectoderm; *ul.p. (mes.)*, anterior end of mesodermal plug; *yc.*, yolk-cell; *p.yb.*, perforated yolk body. Six dividing nuclei are seen. (0.004 mm. thick.)

FIG. 35. Median sagittal section of embryo at stage Fig. 24: *ch.*, chorion; *am.*, amnion; *am.c.*, amniotic cavity, now completely closed; *sc.*, serosal cells; *a.ec.*, anterior ectoderm; *yc.*, yolk-cells; *ul.p.*, mesodermal plug; *mes.*, mesoderm. Seven dividing nuclei in different phases. (0.004 mm. thick.)

FIG. 36. Median sagittal section of embryo, between Figs. 24 and 26, with anterior end square: *sc.*, serosal cells; *s.*, serosa; *am.*, amnion; *am.c.*, amniotic cavity; *a.ec.*, anterior ectoderm; *mes.*, mesoderm; *yc.*, yolk-cell; *yb.*, yolk body. Ten dividing nuclei in different stages. (0.004 mm. thick.)

FIG. 37. Median sagittal section of embryo in Fig. 27 (or 26): *am.p.*, amnion at posterior end; *am.a.*, amnion, thinned out at anterior end; *am.c.*, amniotic cavity; *mes.p.*, mesoderm under posterior end; *mes.*, mesoderm in the middle region; *mes.a.*, anterior limit of mesoderm; *ect.cp.* (anterior ectoderm as in previous figures *a.ec.*), now ectoderm of cephalic region; *p.yb.*, perforated yolk body; *yc.*, yolk-cells, nuclei in large masses of protoplasm. Nine dividing nuclei. (0.004 mm. thick.)

EXPLANATION OF PLATE XXXII.

The outlines of the figures on this plate were drawn with the aid of a camera. They are magnified 55 diameters by Bausch & Lomb's *oc.* 50 mm., *object.* 17 mm.

The series represents, diagrammatically, in side view and partly in optical section, the principal stages in the development of the Termite embryo, from the complete establishment of the germ-disc to the dorsal closure of the body-walls.

The origin and history of the embryonic membranes are particularly emphasized. The general relations of the embryo to these membranes, to the yolk-mass, and to the axes of symmetry of the egg are also well brought out. Note the position of the micropyles, on the primary (and definitive) ventral surface of the posterior end of the egg, in studying the remarkable changes in position which the embryo passes through.

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- STAGE *A*. Germ-disc when first established, *em.*; extra-embryonal blastoderm, *ex.*; yolk, *y.*; chorion, *ch.*; micropyles, *my.*
- " *B*. Germ-disc with posterior amniotic thickening, *am.t.*, and mesodermal plug.
- " *C*. Amniotic fold. Amnion a part of disc, *am.*; serosa, extra-embryonic, *s.*
- " *D*. Amniotic fold just before closure of amniotic cavity. Amniotic cavity open anteriorly, *amc.*; serosa, *s.*
- " *E*. Immediately after closure of amniotic cavity, *amc.*
- " *F*. Early stage in the posterior elongation. The amnion begins to be stretched.
- " *G*. Elongating germ-band before appearance of cephalic lobes. Amnion not fully stretched posteriorly. Mesoderm a flat under-layer.
- " *H*. Unsegmented germ-band with cephalic lobes, *cp.l.*
- " *I*. First appearance of segments. Antennae, *ant.*; mandibular segment, *md.*; first maxillary, *max.¹*; second maxillary, *max.²*; first thoracic, *th.¹*; tail-piece, *ta.*
- " *K*. Further elongation. Addition of second and third thoracic segments, *th.²* and *th.³*; and an indistinct first abdominal, *ab.¹*, from anterior portion of tail-piece. Appearance of labrum, *l.*, and stomodeum, *st.* Folding of head up from yolk.
- " *L*. First stage of caudal flexure. Cephalic and thoracic appendages well marked.
- " *M*. Caudal flexure pronounced. Abdominal segments established. Proctodeum, *pr.*, well developed. Anterior appendages prominent.
- " *N*. Just before "revolution." Head globular and standing off from yolk. Maxillae tri-lobed. Anterior appendages long and beginning to segment. Abdominal appendages prominent. Stomodeum and proctodeum long.
- " *O*. "Revolution." Head slipping up along ventral surface to the anterior pole of the egg. Embryonic membranes, especially serosa, contracting dorsally. Proctodeum, *pr.*, a long tube. Second maxillae moved inward and not seen.

STAGE *P*. Completion of "revolution." Dorsal growth of body-walls. Tracheal stigmata, *tr*. Dorsal organ, *d.o.*, the retracted remnants of embryonic membranes.

- " *R*. Closure of body-walls on the mid-dorsal line complete. Dorsal organ has disappeared within yolk-mass, which is now enclosed in mid-gut. Ventral ganglia shown. As compared with the preceding stage, *P*, this is more truly an optical section, not showing the body-walls except along the boundaries of the body.

BIOGRAPHY.

Henry McElderry Knower was born in Baltimore, Md., Aug. 5, 1868. Preparing for college at the late Mr. Geo. G. Carey's school, he entered the Johns Hopkins University in 1887. He was graduated with the degree of Bachelor of Arts in 1890.

On becoming a candidate for the degree of Doctor of Philosophy, he selected Animal Morphology as his principal subject, with Physiology and Botany as first and second subordinates, respectively.

During his work as a graduate student, he held the positions of Assistant in Histology, Assistant in General Biology, University scholar, and Fellow-in-Zoölogy. In the spring of 1895 he was appointed "Adam T. Bruce-Fellow-in-Zoölogy" for the final year of his candidacy for the Doctor's degree.

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